University of Alberta

Detection, Occurrence and Risk Assessment of *N*-Nitrosamines in Drinking Water

by

Jeffrey William Adrien Charrois ©

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To Theresa and Jack
Wherever you are, that's my home.

Abstract

One of the most successful public health measures ever implemented has been the disinfection of drinking water. Reactions between oxidants (*e.g.* chlorine or chloramines) and source water natural organic matter produce chemical disinfection by-products (DBPs). Since detecting trihalomethanes in 1974, including chloroform—the first recognized DBP in drinking water—regulatory and public health concerns have focused on halogenated DBPs. Identification of specific halogenated DBP species that could reasonably explain observed epidemiological correlations between urinary bladder cancer and chlorinated drinking water exposure have yet to be made. *N*-Nitrosamines are a class of non-halogenated DBPs, which warrant consideration because the "probable" human carcinogen *N*-nitrosodimethylamine (NDMA) is a DBP that has been found in drinking waters throughout North America.

Extraction of ultra-trace concentrations of *N*-nitrosamines from water is an analytical challenge because these compounds are hydrophilic, polar compounds. Successful development of a selective and sensitive analytical method capable of detecting eight *N*-nitrosamines (detection limits: 0.4–1.6 ng/L) was achieved using solid-phase extraction (SPE) coupled with GC/MS, using ammonia positive chemical ionization. NDMA concentrations in drinking waters collected throughout Alberta ranged from non-detectable to 180 ng/L, representing some of the highest reported values in the absence of anthropogenic contamination. Additionally, *N*-nitrosopyrrolidine (up to 4 ng/L) and *N*-nitrosomorpholine (up to 3 ng/L) were reported for the first time as drinking water DBPs.

A survey of utilities serving a majority of Alberta's population prompted modifications to the SPE method, allowing simultaneous extraction of 10 samples.

NDMA was detected in 30% of the 20 utilities surveyed, with two locations exceeding Ontario's Drinking Water Quality Objective of 9 ng/L. Most frequently NDMA occurrence was associated with chloraminating facilities.

Bench-scale disinfection experiments suggested maximum NDMA production occurs near the theoretical monochloramine maximum (1:1 Cl_2:NH_3-N, M:M) in the sub-breakpoint region of the disinfection curve. Treatment conditions that included free-chlorine contact (2 hours) before ammoniation resulted in significant reductions in NDMA formation (up to 93%) compared to no free-chlorine contact time.

NDMA as a DBP does not represent a major route of exposure compared to dietary and commercial product sources. Drinking water risk assessments need to consider comprehensive NDMA exposure scenarios in order to truly protect public health.

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I would like to thank Dr. Richard Bull for pointing out the need to investigate *N*-nitrosamines in drinking water; this has been a truly exciting journey. I am deeply indebted to Dr. Harry Priest (Agilent Technologies Inc.) for all your help regarding GC/MS ammonia PCI applications. Thank-you to everyone so willing to share your experiences in DBP research and especially those involved in helping with *N*-nitrosamine analytical confirmation particularly: Dr. Vince Taguchi and Mr. Dave Fellowes (Ontario Ministry of the Environment); Mr. Patrick Halevy (City of Brantford); Dr. Susan Andrews (University of Waterloo); Mr. Stuart Krasner, Dr. Carrie Guo, Ms. Sylvia Barrett, and Ms. Cordelia Hwang (Metropolitan Water District of Southern California);

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List of Abbreviations:

μg/L – microgram per liter

CDHS – California Department of Health Services

CI – Chemical Ionization

DBNA – *N*-Nitrosodi-*n*-butylamine

DBP – Disinfection By-Product

DCM – Dichloromethane

DENA – *N*-Nitrosodiethylamine

DOC – Dissolved Organic Carbon

DPNA – *N*-Nitroso-*n*-dipropylamine

DPNA-*d14* – [14-^{2}H] *N*-Nitroso-*n*-dipropylamine

EI – Electron Ionization

GC/MS – Gas Chromatography / Mass Spectroscopy

HAA – Haloacetic Acid

HRMS – High-Resolution Mass Spectrometry

ID/SS – Isotope Dilution / Surrogate Standard

IDL – Instrument Detection Limit

IMAC – Interim Maximum Acceptable Concentration

IS – Internal Standard

LLE – Liquid-Liquid Extraction

LRMS – Low-Resolution Mass Spectrometry

MAC – Maximum Acceptable Concentration

MDL – Method Detection Limit

MENA – *N*-Nitrosomethylethylamine

MeOH – Methanol

MS/MS – Tandem Mass Spectrometry

MWDSC – Metropolitan Water District of Southern California

NA – Not Analyzed

ND – Not Detected

List of Abbreviations *continued*:

NDMA – *N*-Nitrosodimethylamine

NDMA-*d6* – [6-^{2}H] *N*-Nitrosodimethylamine

ng/L – nanogram per liter

NMor – *N*-Nitrosomorpholine

NPip – *N*-Nitrosopiperidine

NPyr – *N*-Nitrosopyrrolidine

OMOE – Ontario Ministry of the Environment

PCI – Positive Chemical Ionization

poly- DADMAC – poly- diallyldimethylammonium chloride

Q:T – Qualifier-ion : Target-ion ratio

QA/QC – quality assurance / quality control

RR – Relative Response

RRF – Relative Response Factor

RSD – Relative Standard Deviation

S/N – Signal-to-Noise Ratio

SIM – Selected Ion Monitoring

SPE – Solid Phase Extraction

SS – Surrogate Standard

THM – Trihalomethane

TKN – Total Kjehldahl Nitrogen

UA – University of Alberta

UHP – Ultra-High-Purity

USEPA – United States Environmental Protection Agency

WTP – Water Treatment Plant

Chapter One

General Introduction

1.1 Waterborne Infectious Disease

1.1.1 Global perspective

The United Nations (UN) declared 2005–2015 as the International Decade for Action, “Water for Life”, drawing the world’s attention to the importance of water and water-related issues. According to the World Health Organization’s (WHO) best available estimates, 1.8 million people per year die from diarrhoeal diseases, including cholera (WHO, 2004). Most of these deaths are in developing countries and 90% are children under 5 years of age. The majority of diarrhoeal diseases can be attributed (88%) to unsafe water supplies as well as inadequate sanitation and hygiene. Moreover, the WHO (2004) estimates that in 2002 1.1 billion people (17% of global population) did not have access to improved water resources (household connection, public standpipe, borehole, protected dug well, protected spring, or rainwater collection). Finally, 2.6 billion people (42% of global population) lacked access to improved sanitation (connection to a public sewer, connection to a septic system, pour flush latrine, simple pit latrine, or ventilated improved pit latrine) (WHO, 2004).

Globally, mortality due to diarrhea as a result of exposure to infectious agents accounts for more deaths per year than do AIDS and cancer combined (Craun et al., 1994). To say that Canadians are fortunate to have access to safe drinking water and adequate sanitation is certainly an understatement, particularly when compared to many developing areas throughout the world. However, the potential for source water contamination or drinking water system failure are ever present. Improper waste management, failure to monitor critical control points, and complacency are all real threats that can undermine the ability to deliver safe drinking water, even in developed countries. Indeed, episodes of water-borne outbreaks continue to occur regularly in affluent nations (Hrudey and Hrudey, 2004). Thus, microbial control in drinking water is as important in developing countries as it is in industrialized nations.

1.1.2 Walkerton, Ontario

The beginning of my thesis research coincided with the tragic Walkerton outbreak. In May 2000 drinking water in Walkerton, Ontario became contaminated with *Escherichia coli* O157:H7 as well as *Campylobacter jejuni* (O'Connor, 2002). Without a properly maintained water treatment plant, there was neither adequate disinfection nor chlorine residual to protect against consuming pathogenic microorganisms via drinking water. The consequences of failed disinfection officially accounted for seven deaths and 2300 related illnesses (O'Connor, 2002), with children and the elderly taking the brunt of the effects.

The name "Walkerton" has become synonymous with attributes of incompetence, failure, deceit, and mistrust. Unfortunately, this redefinition of "Walkerton" takes the focus away from what I believe many people have too quickly forgotten or have failed to recognize altogether; that Walkerton is a real place where real people had their lives changed forever. In Chapter 2 of Part One, Report of the Walkerton Inquiry, Justice Dennis O'Connor documents a few select personal stories of Walkerton town residents, providing a record of experiences and highlighting different aspects of the community's suffering.

A short passage is included below from the Report of the Walkerton Inquiry (O'Connor, 2002) as a testament to the brutal personal suffering and loss that those in Walkerton experienced, not only in May of 2000 but for the lifelong pain many continue to endure. The personal account of suffering is meant to be a reminder that providing safe drinking water is a critical activity that requires not only proper technical training but also an understanding of the responsibility this activity carries with it, for when not conducted correctly, the consequences can be catastrophic. No one in Canada expects to die or suffer harm as a result of drinking tap water. The following is a description from a mother, Tracey Hammell, about her two-year-old son Kody who became infected with *E. coli* 0157:H7 and developed hemolytic uremic syndrome (HUS):

> On Friday, Kody suddenly began to vomit. Mrs. Hammell took him inside to change his diaper and noticed that it was bloody. "I couldn't even tell what it was," she said. I had never seen that before."

> On Saturday morning Mrs. Hammell woke up to find Kody violently ill. At 10:00 a.m., she phoned the hospital but was told that the hospital was "backed up" and that she should not come in just yet. "Well, when can I come?" she asked. "He's really sick." The hospital staff told her to wait until 4:00 p.m. and to get fluids into her son in order to prevent dehydration. "Do whatever you have to do to get it in him," they said: "get a syringe."
>
> Mrs. Hammell followed their advice. She got a syringe and "shoved water down his throat." The water may still have been contaminated, but she did not know that. Finally, she phoned the hospital again around 12:30 p.m. and said, "You've got to see him. He's lifeless. His eyes are rolling in the back of his head ... [He has] diarrhea every two minutes. He can't take it anymore." (O'Connor, 2002, pp. 43–44).

In terms of global public health protection, certainly the risks from pathogenic microorganisms are the principal threat that must be addressed by drinking water providers. The consequences of not adequately disinfecting microbial contaminated drinking water are far more clear and immediate than the long-term consequences of lifetime exposures to disinfection by-products (DBPs). However, in spite of uncertainties and incomplete data, the potential health risks from exposures to DBPs cannot be ignored. An Expert Working Group convened by Health Canada in 1997 concluded, the risk of bladder cancer and possibly other cancers from DBP exposures, poses a risk to public health, which was ranked as a moderately important public health issue (Mills et. al., 1998). If real, the relatively low epidemiology cancer risk estimates from lifetime exposures to DBPs could still translate into a significant number of cases at the population level, making DBP research necessary. Assessing human health risks associated with chemical DBPs must be done while considering the larger water quality context. Before costly changes are implemented at a drinking water treatment plant, best available DBP research ought to be incorporated into the risk management process, especially when decisions could impact microbial disinfection efficacy. Making well informed decisions allows for a better balancing of risk-tradeoffs, whether between microbial and chemical risks or between disinfection alternatives.

1.2 Disinfection Chemistry

1.2.1 Chemistry of free chlorine

Use of chlorine remains the most popular and cost effective form of disinfection for drinking water (Craun et al., 1994). Free chlorine is typically used as the primary disinfectant and can be used in combination with other secondary disinfectants such as chloramines, ozone, chlorine dioxide, or ultra-violet radiation. The most common forms of chlorine for use in water treatment plants are chlorine gas and sodium hypochlorite (liquid) (MWH, 2005). When chlorine gas is added to water, it rapidly hydrolyzes to form hydrochloric acid (strong acid) and hypochlorous acid (weak acid) (**Equation 1.1**), which further dissociate (**Equation 1.2** and **1.3**). Sodium hypochlorite hydrolyzes to form hypochlorous and hydroxide ions (**Equation 1.4**).

$Cl_{2\,(g)} + H_2O \rightarrow HCl + HOCl$ **(Equation 1.1)**

$HCl \rightarrow H^+ + Cl^-$ **(Equation 1.2)**

$HOCl \leftrightarrow H^+ + OCl^-$ **(Equation 1.3)**

$NaOCl + H_2O \rightarrow HOCl + Na^+ (OH)^-$ **(Equation 1.4)**

1.2.2 Chemistry of combined chlorine

In the presence of ammonia, a series of reactions take place with chlorine to form chloramines. Ammonia can either be added as part of the treatment process or can be present in raw water sources (naturally or because of anthropogenic contamination). Chloramine species, which are often referred to as combined chlorine, include monochloramine, dichloramine, trichloramine as well as organochloramines. The predominant form of chloramine will vary depending on conditions such as: pH, the chlorine:ammonia ratio, and forms of nitrogen present (White, 1999; MWH, 2005). In terms of disinfection efficiency, monochloramine is the preferred species.

Monochloramine formation: $HOCl + NH_3 \rightarrow NH_2Cl + H_2O$ **(Equation 1.5)**

Dichloramine formation: $NH_2Cl + HOCl \rightarrow NHCl_2 + H_2O$ **(Equation 1.6)**

Trichloramine formation: $NHCl_2 + HOCl \rightarrow NCl_3 + H_2O$ **(Equation 1.7)**

Free chlorine is considered the sum of hydrochloric acid and hypochlorous acid species (**Equation 1.8**). The sum of **Equations 1.5, 1.6** and **1.7** is termed combined chlorine (**Equation 1.9**). The sum of **Equations 1.8** and **1.9** is termed total chlorine (**Equation 1.10**).

Free chlorine = $HOCl + OCl^-$ **(Equation 1.8)**

Combined chlorine = $NH_2Cl + NHCl_2 + NCl_3$ **(Equation 1.9)**

Total chlorine = Free chlorine + Combined chlorine **(Equation 1.10)**

Although chloramines have a lower germicidal efficiency compared to free chlorine, chloramines are frequently used because they: remain longer in the distribution system, are easy to apply, and are simple to detect using established analytical techniques (Kirmeyer et al., 2004). Most importantly from a DBP perspective, use of chloramines generally results in a lower formation of regulated DBPs, specifically THMs (for Canada and the United States) and haloacetic acids (HAAs) (United States). Historically, chloramine use spiked in 1936 (16% of all U.S. water treatment facilities) but declined during World War II because of shortages of ammonia (Kirmeyer et al., 2004). More recently, in an analysis of the Information Collection Rule (ICR) data, 34.7% of the 353 treatment plants examined indicated use of chloramines with some type of chlorine pretreatment and 11.5% used chloramine in combination with ozone or chlorine dioxide pretreatment (McGuire et al., 2002). Interests in the application of alternative disinfectants, such as chloramines, have again been increasing because of regulations related to disinfection by-product concentrations (USEPA, 1998a).

1.2.3 Breakpoint chlorination

Breakpoint chlorination occurs when sufficient chlorine has been added to water containing ammonia, meeting the chlorine demand of the water and oxidizing all of the ammonia, after which point any additional chlorine is measured as a free chlorine residual. An idealized breakpoint chlorination curve, divided into four zones for ease of explanation, is shown in **Figure 1.1**.

Initially, free chlorine added to an authentic system would react with any reducing agents present in the water such as iron, manganese, sulfide, and nitrite (Zone A). Once the initial chlorine demand is met, additional free chlorine begins to form combined chlorine species as ammonia is consumed (Zone B). Graphically, chlorine demand is the difference between the 45° zero chlorine demand line and the measured total chlorine residual line. Monochloramine, which is stable, predominantly occurs in Zone B, with some dichloramine and only traces of trichloramine (at neutral to low pH or at high Cl_2:NH_3 ratios). Chlorinated organic species can also form depending on the species of nitrogen precursors initially present in authentic waters. Total combined chlorine formation peaks at a ratio of approximately 5:1 (Cl_2:NH_3-N mass basis) or 1.0 expressed as a mole ratio (Cl_2:NH_3). Reactions in Zone B occur quickly, reaching a metastable equilibrium within seconds to few minutes, compared to Zones C and D where time to metastable equilibrium is in the several minutes to hour range (MWH, 2005).

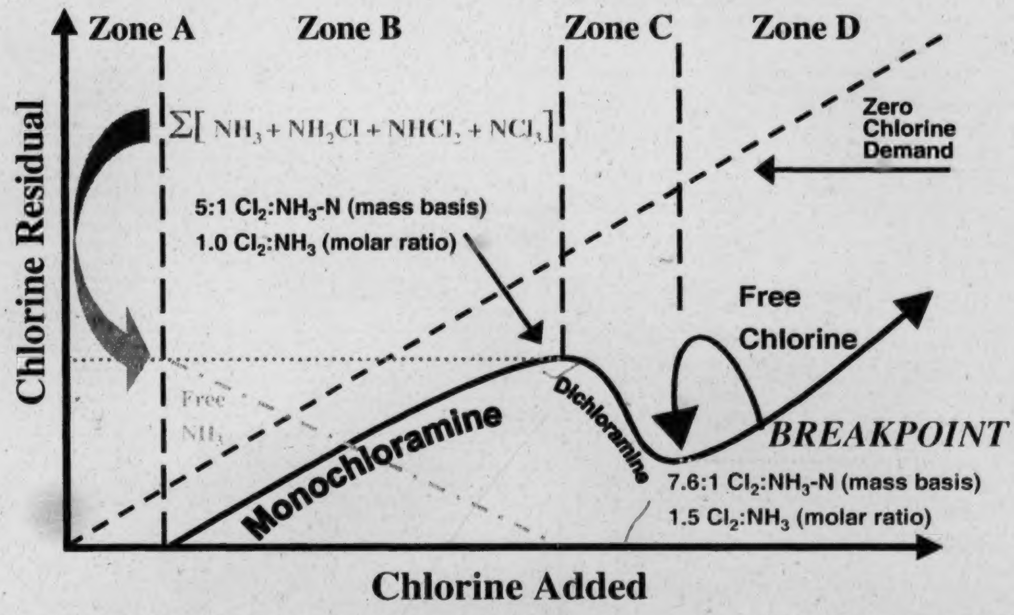

Zone A
Zone B
Zone C
Zone D
$\Sigma[\ NH_3 + NH_2Cl + NHCl_2 + NCl_3\ ]$
5:1 Cl_2:NH_3-N (mass basis)
1.0 Cl_2:NH_3 (molar ratio)
Zero Chlorine Demand
Free Chlorine
Free NH_3
Monochloramine
Dichloramine
BREAKPOINT
7.6:1 Cl_2:NH_3-N (mass basis)
1.5 Cl_2:NH_3 (molar ratio)
Chlorine Residual
Chlorine Added

Figure 1.1. Idealized breakpoint point chlorination curve. This figure is simplified for reactions occurring in pure water between chlorine and ammonia. Authentic water samples will have more complex relationships with less ideal shapes because of the influence of various forms of chlorine-reactive nitrogen other than ammonia. (Figure adapted from: White, 1999; AceOps, 2005; and MWH, 2005).

Beyond Zone B, monochloramine and chlororganic species increasingly become destroyed and the total chlorine residual decreases in spite of the addition of more chlorine. The term disproportionation reaction applies here, where one substance is transformed into two dissimilar compounds in a process that involves simultaneous oxidation and reduction (White, 1999). Additional chlorine begins to oxidize chloramine species and eventually continues until all chloramine species are gone. In Zone C there is a mixture of some monochloramine with dichloramine predominating and possibly trichloramines at low pH. At a theoretical ratio of 7.6:1 (Cl_2:NH_3-N mass basis) or 1.5 expressed as a mole ratio (Cl_2:NH_3), breakpoint occurs. At breakpoint, ammonia nitrogen reaches a minimum and a stable chlorine residual is achieved. From a practical standpoint the exact ratio of chlorine and ammonia resulting in an observed breakpoint may shift and has been observed to occur across a range of values (7:1–16:1; mass basis), Barrett et al. (1985). Factors affecting the location of the breakpoint include: pH, temperature, contact time, chlorine concentration, and the presence of other nitrogen compounds that can react with free chlorine (White, 1999; Kirmeyer, 2004).

After the breakpoint any additional free chlorine added is measured directly as free chlorine residual (Zone D). Note, the term *free chlorine* residual specifies a condition when HOCl is ≥ 85% of the total chlorine residual measured (White, 1999).

The pH of the system has a major influence on the chloramine species that will be present. Even in Zone B, dichloramines can become more significant as the pH drops. Additionally, under low pH conditions, trichloramine may be present in Zone D as part of the stable chlorine residual.

1.3 Chemical Disinfection and Disinfection-Byproducts (DBPs)

1.3.1 Disinfection-byproducts

Disinfection of drinking water is one of the greatest advances in public health protection. However, during the treatment of drinking water, unintended chemical disinfection by-products (DBPs) result from reactions between oxidants used for disinfection (*e.g.* chlorine, chloramine, ozone, or chlorine dioxide) and diverse groups of precursors within source water (*e.g.* natural organic matter (NOM); bromide; and anthropogenic compounds such as amine-based coagulant aids). In general, DBP formation reactions can be generalized as follows:

$$\textbf{Disinfectant + Precursors} \rightarrow \textbf{DBPs} \qquad \text{(Equation 1.11)}$$

Trace concentrations (μg/L) of DBPs in drinking water have been known for over 30 years, ever since chloroform and other trihalomethanes (THMs) were first identified (Rook, 1974; Bellar et al., 1974). Past DBP research has almost exclusively been on chlorinated and brominated species of the two major DBP classes: (1) THMs and (2)

HAAs. Early national drinking water surveys found THMs ubiquitous in chlorinated drinking water throughout the United States (USEPA, 1978). The initial focus on THMs and HAAs was in part because of available analytical techniques to detect these groups of compounds as well as their relatively high abundance in drinking water. Throughout history, identification of DBP species has closely paralleled advances in analytical chemistry. As detection instrumentation and analytical methods improved, so did the ability to identify and quantify new compounds in drinking water. Today, > 500 individual DBP species, representing several chemical classes, have been identified in drinking water (Richardson, 1998). However, the pursuit of lowering chemical analytical detection limits and novel compound identification should not supercede the primary goal of water treatment, that is, production of safe drinking water. Public health concerns surrounding DBPs persist, despite large efforts and millions of dollars being spent on investigating adverse health outcomes and engineering process-control research. Improved DBP research is required now more than ever, to better understand DBP occurrences and to integrate more completely toxicity data with human health outcomes from epidemiology studies.

1.3.2 Alternative disinfectants

"Alternative disinfectants", such as chloramines, ozone, or chlorine dioxide, are increasingly being used in place of, or in combination with chlorination because of the tendency to produce less regulated chlorinated DBPs such as THMs and HAAs (Diehl et al., 2000). Consideration of the upcoming Stage 2 Disinfection Byproducts Rule is a definite regulatory driver compelling more water utilities to incorporate alternative disinfectants in hopes of achieving lower regulated DBP levels. Ironically, attempts to reduce regulated DBP concentrations using alternative disinfection processes have, in some cases, been found to generate higher concentrations of unregulated and more toxic DBPs compared to chlorination alone. Examples of DBPs occurring at higher concentrations when alternative disinfectants are used include: halonitromethanes (pre-ozonation), iodo-THMs (chloramines), and dihaloaldehydes (chloramines and ozone) (Weinberg et al., 2002). Halonitromethanes have been shown to be potent genotoxicants in mammalian cells, several of which are more genotoxic than regulated DBPs (Plewa et al., 2004a). New *in vivo* work is revealing that halonitromethanes also form DNA adducts in rat livers (DeAngelo, 2005). Moreover, iodoacid DBPs have recently been identified in chloraminated drinking waters, which were derived from high bromide and iodide containing source waters (Plewa et al., 2004b). The iodoacid DBPs tested exhibited increased cytotoxicity and genotoxicity compared to bromoacetic and chloroacetic acids, which are regulated HAAs in the United States. Finally, non-halogenated *N*-nitrosamines warrant consideration because the "probable" human carcinogen *N*-nitrosodimethylamine (NDMA) (USEPA, 1987) is a DBP and has been found in drinking waters throughout North America at toxicologically relevant concentrations (ng/L; parts-per-trillion) (Barrett et al., 2003; Charrois et al., 2004).

These newly identified DBP classes, which may be preferentially formed when using alternative disinfectants, are of concern because they present an uncertain level of risk to human and environmental health. This uncertainty is a critical research gap that must be addressed in locations currently utilizing or considering adopting alternative disinfection methods.

1.4 Cancer Epidemiology

Public concerns regarding exposure to DBPs and adverse health outcomes stem from toxicology and epidemiology studies, which have demonstrated elevated risks of developing certain cancers or adverse reproductive outcomes with increased exposure to DBPs in drinking water (Fawell et al., 1997; Arbuckle et al., 2002). Of all forms of cancer investigated in epidemiology studies, urinary bladder cancer appears to be the most biologically plausible for humans to develop from DBP exposures (Mills et al., 1998; Bull et al., 2001). Epidemiology studies can assess risks to exposed human populations, which avoid rodent bioassays and extrapolations from high to low dose. However, disadvantages of the epidemiological method include the lack of statistical power to detect small risks and the difficulty of ensuring accurate exposure assessments (Bull et al., 1995).

Shortly after the 1974 identification of chloroform as well as other chlorinated by-products in drinking water, epidemiology studies began to investigate potential human health risks from exposures to disinfected drinking water. Initial epidemiology studies were ecological in design, meaning exposures and outcomes were assessed at the population level and not directly linked to individuals (Cantor et al., 1978; Kuzma et al., 1977; Bean et al., 1982). Given the aggregated nature of the early epidemiology studies, they could not test for causality, however, there were indications that bladder cancer might be associated with consumption of chlorinated drinking water. Ecologic designs are typically quick, inexpensive, and easy to conduct, relative to other epidemiology study designs and serve to generate future research questions.

A second generation of epidemiology investigations used case-control studies, which had the advantage over ecological studies of incorporating data for individuals (Cantor et al., 1987; Zierler et al., 1988; Lynch et al., 1989; King and Marrett, 1996; Freedman et al., 1997; Cantor et al., 1998). Overall, using the case-control study design is useful to investigate bladder cancer and DBP exposures because case-control studies are most useful when a disease of interest is relatively rare and exposures are common (Gordis, 2000). Gathering information on identified cases is more efficient than in a cohort study design where many subjects must be recruited just to find the disease of interest. Another advantage of the case-control design is that since the disease has already occurred, the researchers do not have to wait for diseases with long latency periods, saving time and money (Fletcher et al., 1996). Case-control studies do have some design limitations. One limitation is the difficulty in selecting appropriate cases and controls (selection bias problems). Most importantly, there are potential biases in the exposure assessment.

The validity of exposure measurements can be questioned because exposure is determined after measuring the disease (measurement and recall biases). Disease state may influence a subject's recollection and lead to an overestimation of exposure. Thus, there is a need for objective measures of assessing exposure. Fawell et al. (1997) have called for improvements in DBP exposure assessment methodology for epidemiology studies. The magnitude of association tended to increase between human exposure to DBPs and an adverse health outcome, such as bladder cancer, when the exposure assessment was improved (King and Marrett, 1996). Additionally cheaper and faster analytical assessments are required, so that epidemiologists can afford to incorporate better measures of exposure in their studies.

A recent meta analysis of primary data obtained from six case-control studies of incident urinary bladder cancer (histologically confirmed), calculated an adjusted odds ratio of 1.24 (95% CI 1.09 – 1.41) for men who were ever exposed to average THM concentrations > 1 μg/L, during a 40 year exposure window, compared to those who had lower or no exposure (Villanueva et al., 2004). Additionally, for men, a dose-response pattern of increasing risk (OR = 1.44; 95% CI 1.20–1.73) with increasing exposure (> 50 μg/L THMs) was measured. Women with the same exposure scenarios showed no increased risk of bladder cancer. Total THM concentrations can be used as a surrogate for chlorinated DBP exposures in epidemiology studies (SENES, 2003; Villanueva et al., 2004), however, at concentrations found in treated drinking water chloroform—typically the most abundant THM—is not capable of producing bladder cancer (USEPA, 2001). Specifically, chloroform is likely to be carcinogenic to humans by all routes of exposure under high-exposure conditions that lead to cytotoxicity and regenerative hyperplasia in susceptible tissues (USEPA, 1998b, 1998c). Therefore, at low concentrations, such as in drinking water, chloroform is not likely to be carcinogenic to humans by any route of exposure under exposure conditions that do not cause cytotoxicity and cell regeneration (USEPA, 2001).

Significant DBP research efforts have yet to elucidate a plausible DBP agent(s) and mechanism of action leading to bladder cancer (Bull et al., 2001). This is in spite of epidemiology studies continuing to find low but consistent associations between increased consumption of disinfected drinking water and chronic adverse outcomes such as urinary bladder cancer. If real, the relatively low epidemiology cancer risk estimates from lifetime exposures to DBPs could still translate into a significant number of cases at the population level. Thus, continued, well-focused DBP research is still necessary. However, new drinking water research must be refocused towards DBPs that are biologically capable of producing the observed adverse outcomes measured in epidemiology and toxicology studies.

1.5 *N*-Nitrosamines

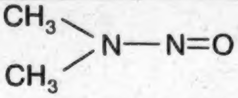
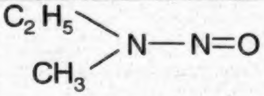
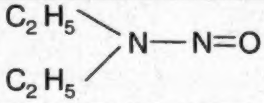
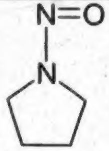
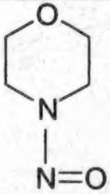
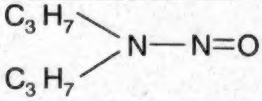
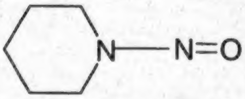
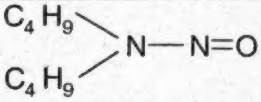
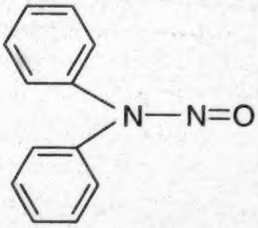
1.5.1 *N*-Nitrosamines: general

The *N*-nitrosamine species of interest throughout this thesis are presented in **Table 1.1**. These constituents were chosen in part because authentic primary analytical standards, as well as select isotopically labeled standards, were available for purchase. *N*-Nitrosodimethylamine (NDMA), the primary focus of this thesis, is the simplest dialkylnitrosamine, characterized by its *N*-nitroso functional group (–N–N=O) and the amine function (–NR_2, where R is H but other alkyl groups can be present).

1.5.2 *N*-Nitrosamines and the water industry

N-Nitrosodimethylamine (NDMA) and other *N*-nitrosamines are emerging as DBPs of great concern to the water industry because many are "probable" human carcinogens (IARC, 1978; USEPA, 1987). As a chemical group, *N*-nitroso compounds are capable of causing cancer in every vital tissue (Shank, 1981). Specifically, *N*-nitroso compounds can cause bladder cancer (Bull, 2001). For example, *N*-nitroso-*n*-butyl-*N*-(4-hydroxybutyl) amine and *N*-nitroso-*n*-butyl-*N*-(3-carboxypropyl) amine are metabolites, resulting from the bioactivation of *N*-nitrosodibutylamine, which can produce tumors in the urinary bladder (IARC, 1978). In 1989, NDMA was first detected as a DBP in Ontario (Andrews and Taguchi, 2000) and interest continued to grow after the 1998 discovery of NDMA in water throughout California.

Table 1.1. Constituents of a standard *N*-nitrosamine mixture.

Compound	Abbreviation	Molecular Mass	Molecular Structure	Formula
N-Nitrosodimethylamine	DMNA *or* NDMA	74.08		$C_2H_6N_2O$
N-Nitrosomethylethylamine	MENA	88.11		$C_3H_8N_2O$
N-Nitrosodiethylamine	DENA	102.14		$C_4H_{10}N_2O$
N-Nitrosopyrrolidine	NPyr	100.12		$C_4H_8N_2O$
N-Nitrosomorpholine	NMor	116.12		$C_4H_8N_2O_2$
N-Nitroso-*N*-dipropylamine	DPNA	130.19		$C_6H_{14}N_2O$
N-Nitrosopiperidine	NPip	114.15		$C_5H_{10}N_2O$
N-Nitroso-*N*-dibutylamine	DBNA	158.24		$C_8H_{18}N_2O$
N-Nitroso-*N*-diphenylamine	DPhNA	198.22		$C_{12}H_{10}N_2O$

1.5.3 *N*-Nitrosodimethylamine metabolism

Metabolic pathway data for NDMA are derived from laboratory animal studies as well as select NDMA conversion studies from human liver preparations (WHO, 2002). Quantitative human metabolic pathway studies for NDMA have not been conducted. However, WHO (2002) did not identify any qualitative differences in NDMA metabolism between humans and laboratory animals. Bioactivation of NDMA is by an activated cytochrome P450 [CYP2E1]-dependent mixed function oxidase system (Haggerty and Holsapple, 1990; Lee et al., 1996). **Figure 1.2** illustrates the two metabolic pathways, hydroxylation and denitrosation, which proceed through a common intermediate radical [$CH_3(CH_2^{\bullet})N{-}N{=}O$] (WHO, 2002). It is through the hydroxylation pathway that the reactive methyldiazonium ion is generated, which strongly alkylates biological macromolecules such as DNA, RNA, and proteins. In terms of DNA adduct formation, N^7-methylguanine (65%) and O^6-methylguanine (7%) are the dominant adduct types (WHO, 2002).

1.5.4 Exposure sources of *N*-nitrosodimethylamine

N-Nitrosodimethlyamine has been detected in tobacco, food (*e.g.* cheeses, smoked meats and canned fruit), beverages (*e.g.* beer, milk and drinking water) as well as cosmetics. In 1992 the Ontario Ministry of the Environment (OMOE) estimated the total daily intake of NDMA from food was 200 ng/day for an average Canadian. In 2002 the World Health Organization (WHO), using primarily Canadian data, projected the daily intake (air, food and water) of NDMA for people aged 20–60 as 250–1100 ng/day. Limited drinking water data exist for NDMA occurrences in drinking water. Typically, NDMA concentrations not affected by anthropogenic contamination are below 10 ng/L, however, concentrations up to 180 ng/L have been reported (Charrois et al., 2004).

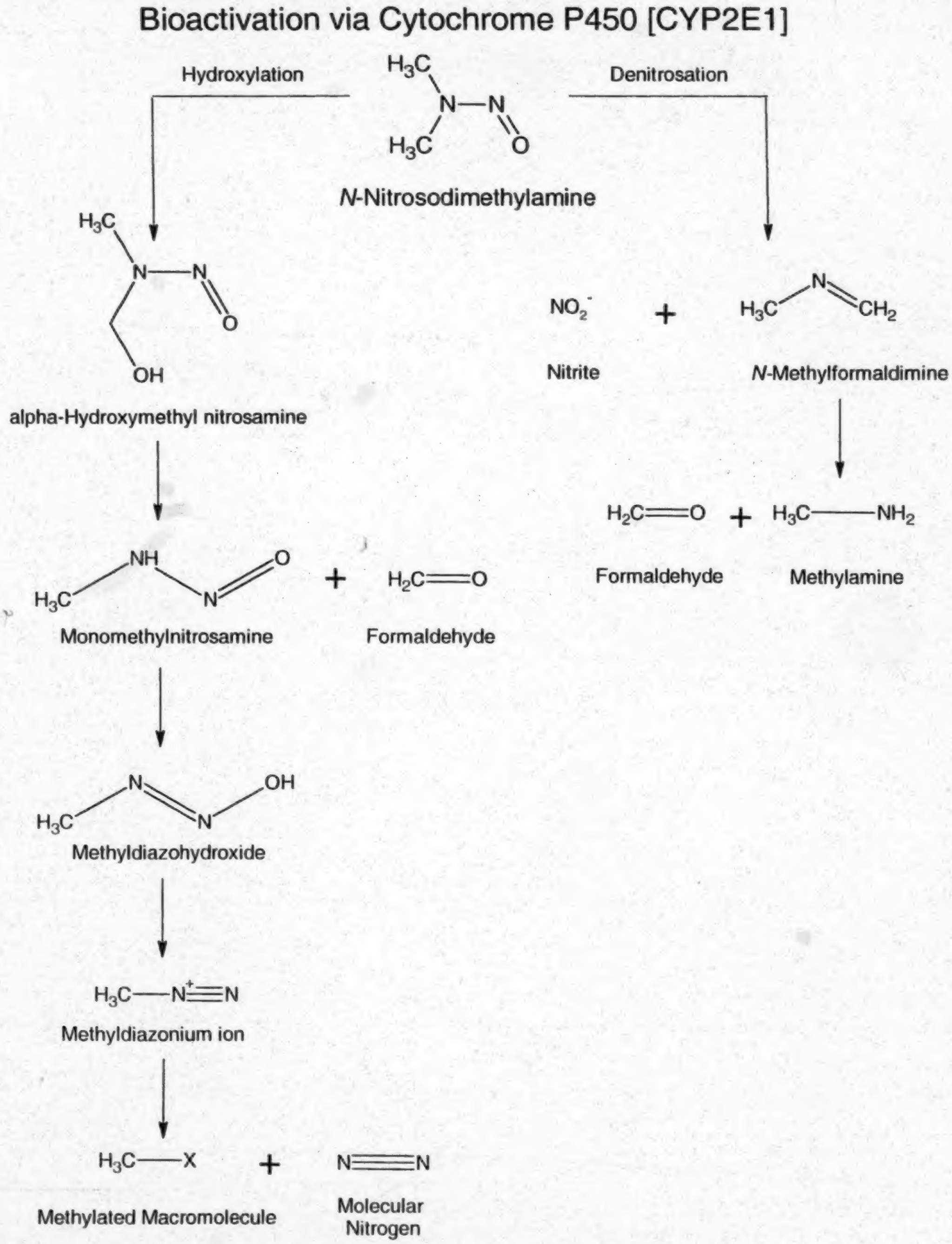

Bioactivation via Cytochrome P450 [CYP2E1]
Hydroxylation
Denitrosation
N-Nitrosodimethylamine
alpha-Hydroxymethyl nitrosamine
Nitrite
N-Methylformaldimine
Formaldehyde
Methylamine
Monomethylnitrosamine
Formaldehyde
Methyldiazohydroxide
Methyldiazonium ion
Methylated Macromolecule
Molecular Nitrogen

Figure 1.2. NDMA metabolic pathway (Adapted from WHO, 2002).

1.5.5 Analytical considerations for *N*-nitrosamines

1.5.5.1 Extraction of N-nitrosamines from water

A major analytical challenge for detecting low concentration (ng/L; part-per-trillion) *N*-nitrosamines involves extraction from water because *N*-nitrosamines are hydrophilic, polar compounds. *N*-Nitrosodimethylamine, for example, is miscible in water (**Table 1.2**). Several methods exist for the extraction of *N*-nitrosamines from water. Liquid-liquid extraction (LLE) is labor intensive and requires the use and disposal of large volumes of solvent, making LLE unattractive for routine use. Solid-phase extraction (SPE) methods for drinking water NDMA are the most commonly used. A wide variety of SPE phases have been used including: Ambersorb® 572 (Taguchi et al., 1994; Jenkins et al., 1995) Carbon disks (Tomkins et al., 1995; Tomkins and Griest, 1996), Envicarb (Cheng et al., 2005)), coconut carbon (USEPA, 2004), as well as a dual phase combination of Ambersorb® 572 and LiChrolut® EN (Charrois et al., 2004).

Table 1.2. Physical-chemical properties of *N*-nitrosodimethylamine (NDMA).

Property	Value	Reference
Water solubility	∞ (miscible)	Mirvish *et al.* (1976)
	∞ (miscible)	Mabey *et al.* (1981)
	1.4E7 mg/L (25C)	Dixon and Rissman (1985)
Octanol-Water (Log P)	0.21	Mabey *et al.* (1981)
	-0.74	Dixon and Rissman (1985)
Henry's Constant	1.82E-6 atm-m^3/mole (37C)	Mirvish *et al.* (1976)
	3E-8 atm-m^3/mole (25C)	Dixon and Rissman (1985)
Vapor Pressure	2.1 mm Hg (20C)	Klein (1982)
	4.87 mm Hg (25C)	Dixon and Rissman (1985)

1.5.5.2 Detection of N-nitrosamines

Analytical methods for the determination of *N*-nitrosamine concentrations in water vary as much as the extraction techniques. Most analytical methods for *N*-nitrosamines have used gas chromatography coupled with a variety of detection systems such as: thermal energy analyzer (TEA) (Fine et al., 1975a and 1975b) and chemiluminescent nitrogen detector (CLND) (Tomkins et al., 1995; Tomkins and Griest, 1996). Additionally, many methods have employed a range of mass spectrometry (MS) techniques such as: i) low-resolution electron ionization (EI) MS (Choi and Valentine, 2002; Kohut and Andrews, 2003); ii) high-resolution electron ionization (EI) MS (Taguchi et al., 1994; Jenkins et al., 1995); iii) chemical ionization (CI) tandem MS with methanol reagent gas (ion trap CI/MS/MS) (Plomley et al., 1994; Mitch and Sedlak, 2002); as well as iv) positive chemical ionization (PCI) with ammonia reagent gas (Charrois et al., 2004).

1.6 Scope of Thesis

Events such as the 1854 cholera outbreak in London, England; the 1993 *Cryptosporidium* outbreak in Milwaukee, WI; and more recently the 2000 *Escherichia coli* O157:H7 contamination event in Walkerton, ON, illustrate the absolute requirement of microbial disinfection in drinking water. Drinking water providers must ensure safety from pathogenic organisms through informed disinfection practices, however, disinfection of drinking water is not risk-free. The risks associated with no treatment, however, are certainly much greater and immediate compared to the uncertain long-term effects of DBP exposure. Ultimately disinfection must continue, resulting in ongoing risk management decisions between both microbial and chemical DBP risk trade-offs as well as trade-offs between various disinfection methods.

Over 30 years of preoccupation with halogenated DBPs, particularly THMs and HAAs, has resulted in an extensive body of literature in the area of drinking water disinfection. Although THMs and HAAs are typically the most frequently occurring DBPs in systems that chlorinate, individual DBP species within these classes are not capable of producing the measured adverse outcomes seen in cancer epidemiology studies. Therefore, I have chosen to investigate *N*-nitrosamines, an emerging class of DBPs, which are 2 to 4 orders of magnitude more potent than regulated halogenated DBP species, specifically THMs and HAAs. In terms of understanding the significance of detecting NDMA and other *N*-nitrosamines in drinking water, research is only just beginning to address some of the key issues.

Utilities included in this study were exclusively from Alberta. In terms of Canadian research, this is the first major work on *N*-nitrosamines as DBPs outside of Ontario, to my knowledge. My research objectives were: (1) to develop an analytical method capable of detecting ultra-trace (ng/L; part-per-trillion) concentrations of *N*-nitrosamines in water (**Chapter 2**); (2) to apply the analytical method to determine the scope of NDMA occurrence by surveying 20 Alberta public drinking water distribution systems (**Chapter 3**); (3) to identify full treatment plant- and bench-scale factors that promote or reduce the formation of NDMA in drinking water, for example, the influence of Cl_2:NH_3-N ratios and free-chlorine contact time during chloramination (**Chapter 4**); and (4) to evaluate lifetime human health cancer risks posed by exposures to *N*-nitrosamines, particularly focused on NDMA (**Chapter 5**). Conclusions and a synthesis of results are presented in **Chapter 6**, which also includes chapter summaries, implications of my research as well as suggestions for future research. Ultimately my research moves towards providing better information for risk management decisions through the investigation of DBP species (*N*-nitrosamines) that are more toxicologically potent compared to currently regulated DBPs (THMs and HAAs).

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Chapter Two

Detecting *N*-Nitrosamines in Drinking Water at Nanogram per Liter Levels Using Ammonia Positive Chemical Ionization[1]

2.1 Introduction

Occurrence of *N*-nitrosamines in source water, wastewater and finished drinking water is an emerging issue of environmental and public health significance because many *N*-nitrosamines are "probable" human carcinogens (IARC, 1978; USEPA, 1987). One *N*-nitrosamine in particular, *N*-nitrosodimethylamine (NDMA), has been detected in drinking water and wastewater after chlorination (Mitch and Sedlak, 2002a) and chloramination (Najm and Trussell, 2001; Choi and Valentine, 2002). In 1989, as part of a surveillance program, NDMA was first detected as a disinfection by-product (DBP) in drinking water in Ontario, Canada (Jobb et al., 1994; Taguchi et al., 1994). Interest in NDMA as a DBP continued to increase after its 1999 discovery in drinking waters and wastewaters throughout California (CDHS, 2004).

Currently, there are no standard analytical methods for *N*-nitrosamines in drinking water at ng/L levels[2]. With the possibilities of future regulation, increased surveys and monitoring, as well as research into formation reactions of NDMA and other *N*-nitrosamines in water, there is increased pressure to develop an improved analytical method. An ideal method would be reliable, selective and sensitive for a range of *N*-nitrosamines in different water matrices as well as be economically viable, in terms of both material costs and time inputs. With several varied analytical approaches available for measuring low level concentrations of NDMA in water, the California Department of

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[1] A version of this chapter has been published. Charrois, J.W.A., Arend, M.W., Froese, K.L., and Hrudey, S.E. 2004. *Environmental Science & Technology* **38:** 4835-4841.

[2] At the time the version of this chapter was published there was no standard method, however, in September 2004 the USEPA published Method 521: *Determination of Nitrosamines in Drinking Water by Solid Phase Extraction and Capillary Column Gas Chromatography with Large Volume Injection and Chemical Ionization Tandem Mass Spectrometry (MS/MS).*

Health Services developed a series of criteria to evaluate data acceptability and reliability, which can be met by the method described herein (CDHS, 2004).

The first analytical challenge for detecting low level (ng/L range) *N*-nitrosamines is the extraction from water because *N*-nitrosamines are hydrophilic, polar compounds. Liquid-liquid extraction (LLE) is very labor intensive and requires the use and disposal of large volumes of solvent, making LLE unattractive for routine use. As an alternative method, solid-phase extraction (SPE) is often employed because of lower costs, shorter processing times, higher sample throughput and ease of automation (Poole, 2003). Low *N*-nitrosamine recoveries can occur with both LLE and SPE methods (Taguchi et al., 1994; Jenkins et al., 1995) and thus an improved extraction method is needed.

Many existing NDMA methods for water samples use isotope dilution quantification methods and are supported by gas chromatography / mass spectroscopy (GC/MS) using electron ionization (EI) (Taguchi et al., 1994; Jenkins et al., 1995; Raksit and Johri, 2001), which lack selectivity and produce potentially non-distinctive fragmentation patterns. High-resolution mass spectrometry (HRMS) can be used to compensate for the lack of EI selectivity in low-resolution mass spectrometry (LRMS).

Positive chemical ionization (PCI) is a “softer” ionization process, resulting in less molecular fragmentation. Methanol reagent gas is commonly used in PCI mode, coupled with ion trap MS/MS for detecting NDMA in drinking water and wastewater samples (Mitch and Sedlak 2002a, 2002b). Through the choice of reagent gas, however, selective ionization can be achieved resulting in lower background noise levels and increased analyte sensitivity (Prest, 1999). Using ammonia reagent gas, adduct formation in the gas phase is favored for amine and nitrosamine groups, compared to methanol PCI, because of similar proton affinities between analytes and reagent gas. Ammonia PCI adduct formation provides increased selectivity compared to LRMS-EI or -methanol PCI, which do not readily form adducts. Furthermore, selectively obtained $[M+18]^+$ and $[M+1]^+$ ions in ammonia PCI mode are more distinctive for quantification compared to the common, low mass fragments produced by EI in complex water matrices (e.g. m/z = 42 and 43 as NDMA EI-qualifier ions) (Prest and Herrmann, 1999).

The main objectives of this work were to: (1) explore the development of a selective, sensitive and affordable bench-top analytical method for detecting several *N*-nitrosamines at relevant drinking water concentrations (low ng/L range) and (2) characterize *N*-nitrosamines from authentic drinking water samples. As used in this thesis, authentic drinking water refers to samples collected from the water treatment plants or distribution system locations under typical operating conditions. Authentic drinking water samples were not subjected to extreme disinfection conditions. Our SPE method combined with GC/MS ammonia PCI was capable of quantifying eight *N*-nitrosamines. Results indicate the high performance of this method for all *N*-nitrosamines tested, as evident from low detection limits (0.4–1.6 ng/L), stable MS-performance (linear-response) and high analyte recoveries. Finally, applying our method to authentic drinking water samples with dissolved organic carbon concentrations (DOC) of 9 mg/L, we were able to detect NDMA concentrations ranging from 2 to 180 ng/L. Furthermore, *N*-nitrosopyrrolidine (NPyr) (2–4 ng/L) and *N*-nitrosomorpholine (NMor) (1 ng/L) were also detected in selected samples, two *N*-nitrosamines not reported in drinking water to date.

2.2 Experimental Section

2.2.1 Materials

Hexane, acetone and *N*-nitrosamine-free reagent water (Optima Grade) as well as dichloromethane (GC-Resolv) were obtained from Fisher Scientific (Nepean, ON, Canada). Methanol (Omni-Solv) was purchased from VWR International (Mississauga, ON, Canada). A standard solution containing nine *N*-nitrosamines was purchased from Supelco (Oakville, ON, Canada) and isotopically labeled standards (98%) ([6-^{2}H] *N*-nitrosodimethylamine, NDMA-*d6* and [14-^{2}H] *N*-nitroso-*n*-dipropylamine, DPNA-*d14*) were from Cambridge Isotope Laboratories (Andover, MA). The SPE materials, Ambersorb® 572 (Rohm & Haas; Philadelphia, PA) and LiChrolut® EN (Merck; Darmstadt, Germany) were supplied through Supelco and VWR International respectively. Ambersorb® 572 consists of carbonaceous spherical beads (particle size: 300–850 μm; surface area: 1100 m^2/g) produced by pyrolysis of sulfonated-styrene divinylbenzene ion-exchange resin, while LiChrolut® EN is an ethylvinylbenzene-divinylbenzene sorbent powder material (40–120 μm; 1200 m^2/g). Sodium bicarbonate (ACS Reagent grade) and L-ascorbic acid (min 99.0%) were obtained from Sigma-Aldrich (Oakville, ON, Canada).

2.2.2 Chemical safety

N-Nitrosamines are suspected human carcinogens, all necessary health and safety precautions should be taken when handling samples containing or suspected of containing these compounds.

2.2.3 Sample collection and quality control

Source-water and treated-water samples from various locations were collected in amber-glass bottles with PTFE lids. At the time of collection, sample bottles were rinsed with a small quantity of sample and the rinsate discarded. Sample bottles were then filled to zero headspace and 20 mg/L of L-ascorbic acid added. Samples were kept cool and transported to the laboratory, where they were refrigerated at 4 °C until the time of extraction, typically within 2 weeks. Trip blanks consisting of reagent water and 20 mg/L of L-ascorbic acid were always included in the sampling procedure. The blanks were handled like samples, transported to the field and returned to the laboratory for analysis. All glassware and sample bottles were washed, rinsed with reagent water, acetone rinsed and baked at 400 °C overnight. Solvent blanks were analyzed for contamination by GC/MS ammonia PCI.

2.2.4 Preparation of stock-standard and calibration-standard solutions

Purchased standard solutions were diluted with methanol to produce stock-standard solutions (mixed *N*-nitrosamine and isotopically labeled) over a range of concentrations (pg/μL). Stock-standard solution dilutions were made on a mass basis. Calibration-standard solutions were produced by volumetrically spiking reagent water with stock-standard solutions of *N*-nitrosamines and NDMA-*d6* (surrogate standard (SS)).

2.2.5 Solid-phase extraction (SPE)

Analytes of interest were extracted using a combination of two SPE materials, which were packed into customized all-glass extraction apparatuses. The extraction apparatus consisted of a 500 mL round-bottom sample reservoir with a modified 12 x 80 mm stem containing a glass frit (40-100 μm pore size) and a 14/20 glass-joint that connected to a vacuum adaptor and collection flask. The top of the flask was connected to a drying-tube (29/40 glass-joint) packed with activated carbon, which assisted in reducing any potential interfering contaminants from laboratory air. In the SPE column, we packed: 350 mg of LiChrolut® EN (bottom), followed by 500 mg of Ambersorb® 572 (middle) and glass wool (top). The SPE bed was pre-cleaned by passing through 15 mL each of hexane and then dichloromethane. Residual solvent was removed by applying a vacuum. The SPE materials were then conditioned with 15 mL of methanol followed by 15 mL of reagent water. Once methanol was added, the SPE bed was not allowed to dry before extraction of the samples was complete.

To each 500 mL sample, 1 g of $NaHCO_3$ (pH ca. 8) and 25 ng of, NDMA-*d6* (SS) were added. Samples were then passed through the SPE bed at a flow rate of 1 drop/sec (3–5 mL/min; total time ca. 2h) under a slight vacuum. Upon completion, a full vacuum (-30 kPa) was applied until the SPE bed was dry. Thorough drying of the SPE phases was critical for proper analyte elution.

Analytes were eluted from the SPE bed with dichloromethane (15 mL) and collected in glass tubes where the internal standard (IS) DPNA-*d14* (25 ng) was added. An additional elution (15 mL) of the SPE materials was also made, to ensure sufficient analyte desorption was achieved from the first elution. Prior to solvent evaporation, 500 μL of methanol was added to each tube. The extracts were slightly heated (40 °C) in an aluminum heating-block and concentrated under a stream of ultra-high-purity (UHP) N_2 to a final volume of 200 μL, resulting in an extract concentration factor of 2500. Extracts were either analyzed immediately or stored at 4 °C prior to GC/MS analysis.

2.2.6 GC/MS ammonia positive chemical ionization (PCI)

N-Nitrosamines were chromatographically separated using an Agilent Technologies (Palo Alto, CA) 6890N gas chromatograph coupled with a 5973 mass selective detector (MSD), operating in PCI mode, with ammonia as the reagent gas. Samples were injected using a Combi PAL (CTC Analytics; Zwingen, Switzerland) autosampler. A J&W Scientific (Palo Alto, CA) DB-1701P capillary column, 30.0 m × 0.25 mm i.d. and 0.25 μm film thickness, was used in combination with the following oven temperature program: initial temperature 40 °C, held for 3 min; then ramp, 4 °C/min to 110 °C; followed by a final ramp of 15 °C/min to 220 °C, held for 2 min (total run time = 34.83 min). The carrier gas, UHP helium, was in constant flow mode at 1.3 mL/min with the gas saver off. The capillary inlet (230 °C) housed a 4 mm deactivated single-taper liner (Supelco). Injection volume was 1 μL, in splitless mode.

The ammonia reagent gas–mass flow controller–was set to 10% (0.5 mL/min). Condensation of ammonia gas must be prevented in the ionization chamber to avoid negative peaks in chromatograms. Therefore, vertical coiling of the reagent gas transfer line may be necessary (Prest et al., 2000). We found that in addition to vertical coiling, an electrical heating band (ca. 60 °C) wrapped around the external reagent gas transfer line before entry into the MSD was essential for eliminating ammonia condensation. In ammonia PCI mode, the GC/MSD interface was 200 °C, the MSD ionization source temperature was set at 200 °C, and the quadrupole at 100 °C. Analytical data were acquired from the MSD in full scan (55–300 m/z) and selected-ion monitoring (SIM) modes. **Table 2.1** contains a list of the target- and qualifier-ions used in SIM mode for ammonia PCI.

2.2.7 Calibration

2.2.7.1 Isotope dilution / surrogate standard procedure.

As used in this paper, isotope dilution / surrogate standard (ID/SS) procedure refers to the determination of *N*-nitrosamine concentrations directly from individual calibration curves. A constant quantity of NDMA-*d6* (25 ng) was spiked at the beginning into calibration-standard solutions containing nine *N*-nitrosamine species, at five concentration levels (9.4 ng/L to 180 ng/L). The calibration-standard solutions were extracted and analyzed for all *N*-nitrosamine species, including the *N*-nitrosamine isotopes. Calibration curves and relative responses (RRs) were calculated for target-ions on an area ratio basis (*N*-nitroso species / NDMA-*d6*), which were plotted against known *N*-nitrosamine concentrations (ng/L). For NDMA this procedure was truly a quantitative determination by isotope dilution. For the other *N*-nitrosamine species it was a surrogate standard procedure with the assumption that NDMA-*d6* was an appropriate surrogate standard for the remaining *N*-nitrosamine species.

2.2.7.2 Internal standard procedure

The internal standard (IS) procedure involved direct GC/MS ammonia PCI analysis of stock-standard solutions (1.8 to 210 pg/μL), which contained: nine *N*-nitrosamines, DPNA-*d14* and NDMA-*d6*. Area responses were used to calculate relative response factors (RRFs) for each compound. The internal standard (DPNA-*d14*) was the basis for determining RRFs for NDMA-*d6* and for the analytes of interest. Relative standard deviations (RSDs), for each RRF, over a range of concentrations were ≤ 10%, so an average RRF was determined for each *N*-nitrosamine species and used for quantification. Internal standard (DPNA-*d14*) was added to each extract prior to GC/MS analysis. The purpose of calculating recoveries for NDMA-*d6* and spiked analytes was to monitor method performance from the start of sample preparation, through extraction, to analysis.

For each GC/MS analysis sequence, a series of standard solutions (for ID/SS and IS) were analyzed before and after authentic samples to ensure a stable instrument response.

2.3 Results and Discussion

2.3.1 Gas chromatography / mass spectrometry (GC/MS)

Gas chromatographic resolution of all *N*-nitrosamine species within calibration-standard mixtures was accomplished (**Figure 2.1**). Complete chromatographic separation of *N*-nitrosamine species from their isotopically labeled analogues was achieved for DPNA and DPNA-*d14* but not for NDMA and NDMA-*d6*. The standard *N*-nitrosamine mixture contained *N*-nitrosodiphenylamine (DPhNA), which decomposed into diphenylamine at high temperatures within the GC injection port (Eichelberger et al., 1983; Ho et al., 1990) and the GC/MSD interface. Since DPhNA adsorbs easily to glass surfaces and because of its thermal instability, it was not considered in this method.

Different fragmentation patterns exist for analytes depending on the reagent gas used in chemical ionization. Formation of ammonia-adducts with quasi-molecular ions at m/z $[M+18]^+$ were the most abundant peaks obtained for all *N*-nitrosamines using ammonia PCI. Protonated molecular-ions $[M+1]^+$ were the second most abundant ions observed. There are few published mass spectra for *N*-nitrosamines using ammonia PCI. Fragmentation pattern data, however, are critical for confirming the identity of analytes when using MS data. For ammonia PCI conditions, DPNA and DPNA-*d14* were used to elucidate general fragmentation patterns for the *N*-nitrosamines of interest. Actual experiments that collected full scan mass spectra for DPNA and DPNA-*d14* stock-standard solutions were conducted, demonstrating ammonia adduct target-ions and protonated molecular-ions of m/z 148 and 131 for DPNA and m/z 162 and 145 for DPNA-*d14* (**Figure 2.2**). Significant fragments existed at m/z 117 (**structure A**) and 131

Table 2.1. ***N*-Nitrosamine species monitored using GC/MS in ammonia positive chemical ionization mode (PCI).**

Compound	Abbreviation	Molecular Formula	Exact Molecular Mass (g/mol)	Monitoring Ions[a] (m/z) Target $[M+18]^+$	Monitoring Ions[a] (m/z) Qualifier $[M+1]^+$
N-Nitrosodimethylamine	NDMA	$C_2H_6N_2O$	74.048	92	75
[6-^{2}H] *N*-Nitrosodimethylamine	NDMA-*d6*	$C_2D_6N_2O$	80.086	98	81
N-Nitrosomethylethylamine	MENA	$C_3H_8N_2O$	88.064	106	89
N-Nitrosodiethylamine	DENA	$C_4H_{10}N_2O$	102.079	120	103
N-Nitrosopyrrolidine	NPyr	$C_4H_8N_2O$	100.064	118	101
N-Nitrosopiperidine	NPip	$C_5H_{10}N_2O$	114.079	132	115
N-Nitrosomorpholine	NMor	$C_4H_8N_2O_2$	116.059	134	117
N-Nitroso-*n*-dipropylamine	DPNA	$C_6H_{14}N_2O$	130.111	148	131
[14-^{2}H] *N*-Nitroso-*n*-dipropylamine	DPNA-*d14*	$C_6D_{14}N_2O$	144.198	162	145
N-Nitrosodi-*n*-butylamine	DBNA	$C_8H_{18}N_2O$	158.142	176	159

[a] Ions used for selected-ion monitoring, ammonia PCI.

(**structure D**) for DPNA and DPNA-*d14* respectively. Structures A and D resulted from the elimination of hyponitrous acid HNO (Δ m/z 31) from the ammonia adduct target-ions, forming 2,2-dipropyl–hydrazinium-ions $(C_3H_7)_2NNH_3^+$ (m/z 117) and $(C_3D_7)_2NNH_3^+$ (m/z 131). Corresponding hydrazinium ions were observed in the full scan mass spectra of all investigated *N*-nitrosamines when using ammonia PCI. Ammonium-ion analogues (**structures B and** E) and immonium-ion analogues (**structures C and** F) were formed for all *N*-nitrosamines investigated. Comparing DPNA and DPNA-*d14* mass spectra, a difference of 14 mass units was measured between the corresponding ammonium-ions (m/z 102 (**structure B**) and m/z 116 (**structure** E), indicating the presence of all 14 deuteriums in the [14-^{2}H]-dipropyl-ammonium-ion fragment. When considering the two immonium-ion fragment ions at m/z 100 (**structure C**) and m/z 113 (**structure F**) in the mass spectra of DPNA and DPNA-*d14*, the measured mass difference was m/z=13, between the labeled and unlabeled species. A Δ m/z=13 suggests the difference was due to one less deuterium. Similar fragmentation patterns were noted for all eight investigated *N*-nitrosamines (heterocyclic and aliphatic).

In this study, ammonia PCI full scan mass spectra were also collected for select authentic drinking water samples. **Figure 2.3** shows an ammonia PCI full scan mass spectrum containing NDMA from an extracted authentic drinking water sample (71 ng/L) compared with matching fragments from the mass spectrum of an NDMA stock-standard solution (250 pg/μL). Using the SPE extraction procedure coupled with GC retention times, qualifier- and target-ion area ratios and MSD spectral data (full scan and SIM), it was possible to confirm the presence of NDMA in authentic drinking water samples. Presence of other *N*-nitrosamines such as NPyr and NMor in authentic samples was confirmed by GC retention times and qualifier- and target-ion area ratios acquired in SIM-mode, only.

2.3.2 – Evaluation of ammonia PCI performance

The consistent formation of ammonia-adduct and protonated-molecular ions (target and qualifier) over a range of concentrations was essential for obtaining linear responses and for giving reproducible results for reliable *N*-nitrosamine quantification. This was reflected in a relatively constant area ratio between the abundance of qualifier-ions and target-ions (Q:T) as well as by a linear fit for the calibration points. In the quantification

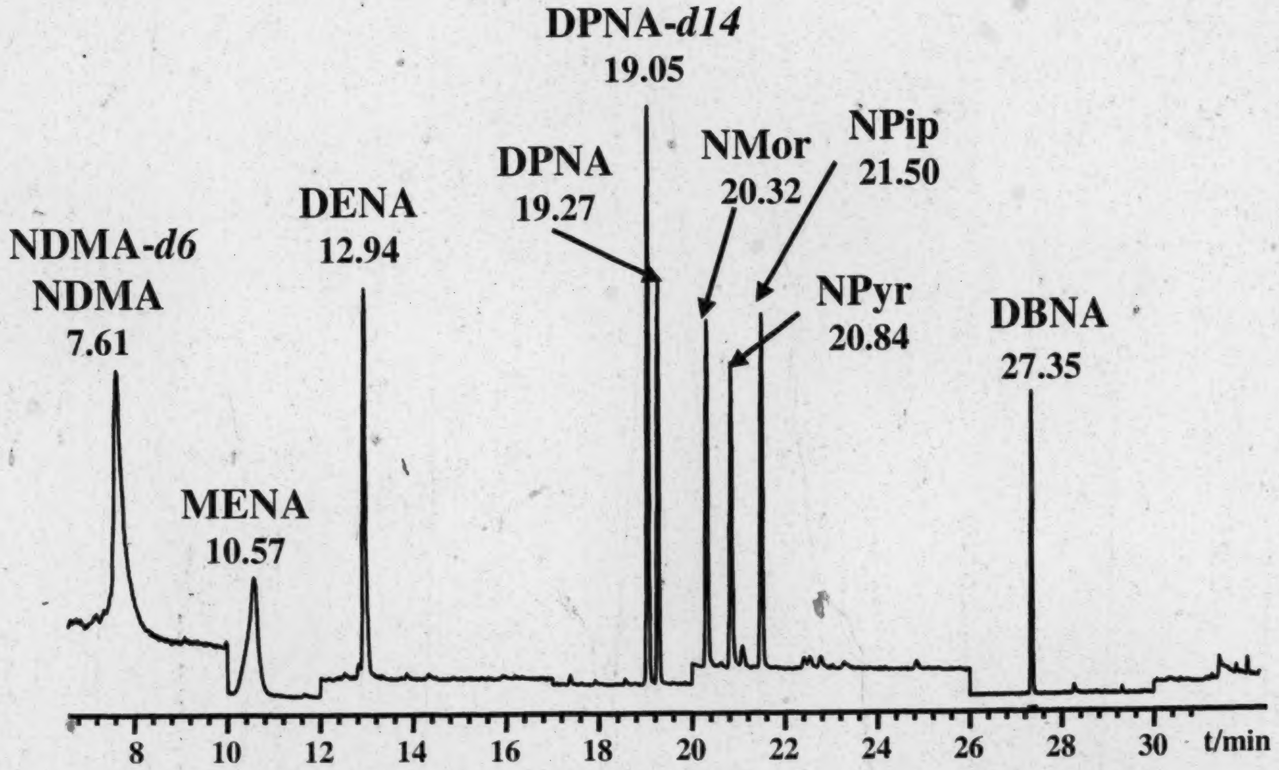

Figure 2.1. Chromatographic separation of *N*-nitrosamine and isotopically labeled species from an extracted calibration-standard mixture (40 ng/L). Total ion chromatogram collected in selected-ion monitoring mode using a DB-1701P column, 1 μL injection. Retention times are given below each compound abbreviation. See Table 2.1 for abbreviation descriptions.

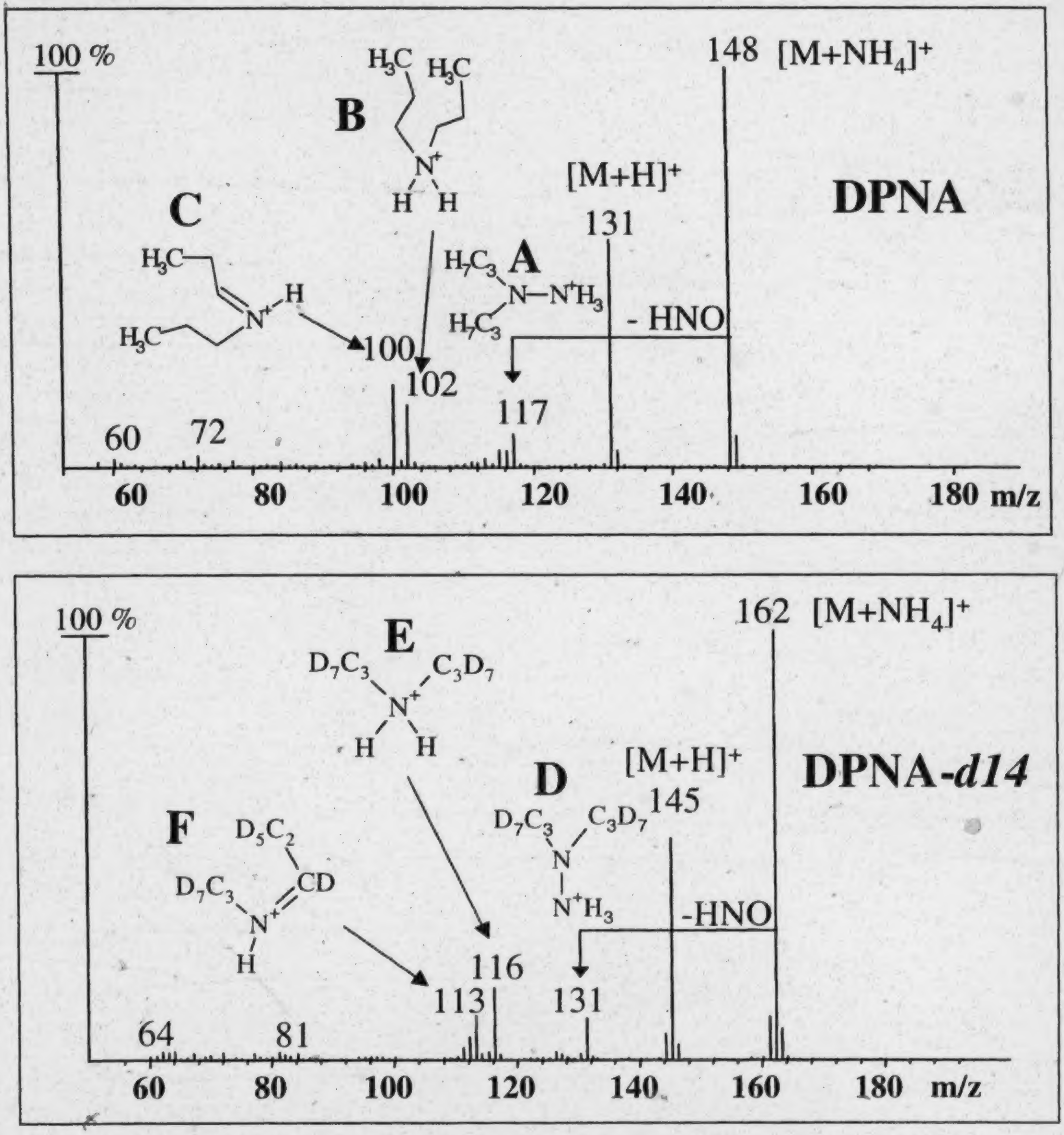

Figure 2.2. Full scan, ammonia positive chemical ionization, mass spectral comparison of *N*-nitroso-*n*-dipropylamine (DPNA) and [14-^{2}H] *N*-nitroso-*n*-dipropylamine (DPNA-*d14*), 1µL injection of a 250 pg/µL stock-standard solution.

process, Q:T stability was critical because it was one criterion used with authentic drinking water samples when identifying unknown chromatographic peaks as truly being *N*-nitrosamines of interest. The Q:T ratio for NDMA was the peak area of m/z 75 divided by the peak area of m/z 92. *N*-nitrosamine stock-standard solutions (2–200 pg/μL; n=8) as well as extracts from calibration-standard solutions (10–200 ng/L; n=5) were assessed for Q:T stability (n=7). *N*-Nitrosamine Q:T ratios, including isotopic standards, are presented in **Table 2.2**. The Q:T ratios remained consistent for both *N*-nitrosamine stock-standard solutions as well as those for SPE extracted calibration-standard solutions. The RSDs of the Q:T, for the *N*-nitrosamines of interest, ranged from 1% to 5%. Using different instrumental conditions, the Q:T ratio will change, however, it is the stability of the ratio for a specified set of conditions during a defined period of time (or set of samples) that is important for evaluating method performance.

2.3.3 Isotope dilution / surrogate standard (ID/SS) and internal standard (IS) calibration

All *N*-nitrosamine concentrations were quantified using GC/MS ammonia PCI using ID/SS and IS procedures. For all eight *N*-nitrosamine species using the ID/SS procedure, R^2-values (n=5) ranged from 0.9960 for DBNA to 0.9996 for MENA **(Table 2.2)**. For IS method evaluation, average RRFs and RSDs were calculated for stock-standard solutions **(Table 2.2)**. The RRFs may change over time due to instrument fluctuations so stock-standard solutions were analyzed in every sequence containing authentic samples. The main consideration was whether there was low variation within RRFs for a given analytical sequence. The RSDs (n=8) of average RRFs varied from 3% for NMor to 11% for NPyr. Overall, R^2 values for ID/SS were near 1.0 and RRFs were consistent for IS, within the investigated concentration ranges, indicating stable method performance for eight *N*-nitrosamines.

Average accuracies (%) for the eight spiked *N*-nitrosamines were evaluated using ID/SS **(Table 2.3)**. The average accuracies using extracted calibration-standard solutions (10–200 ng/L; n=5) ranged from 94% for NPyr to 102% for *N*-nitrosopiperidine (NPip). The RSDs were all less than 10%.

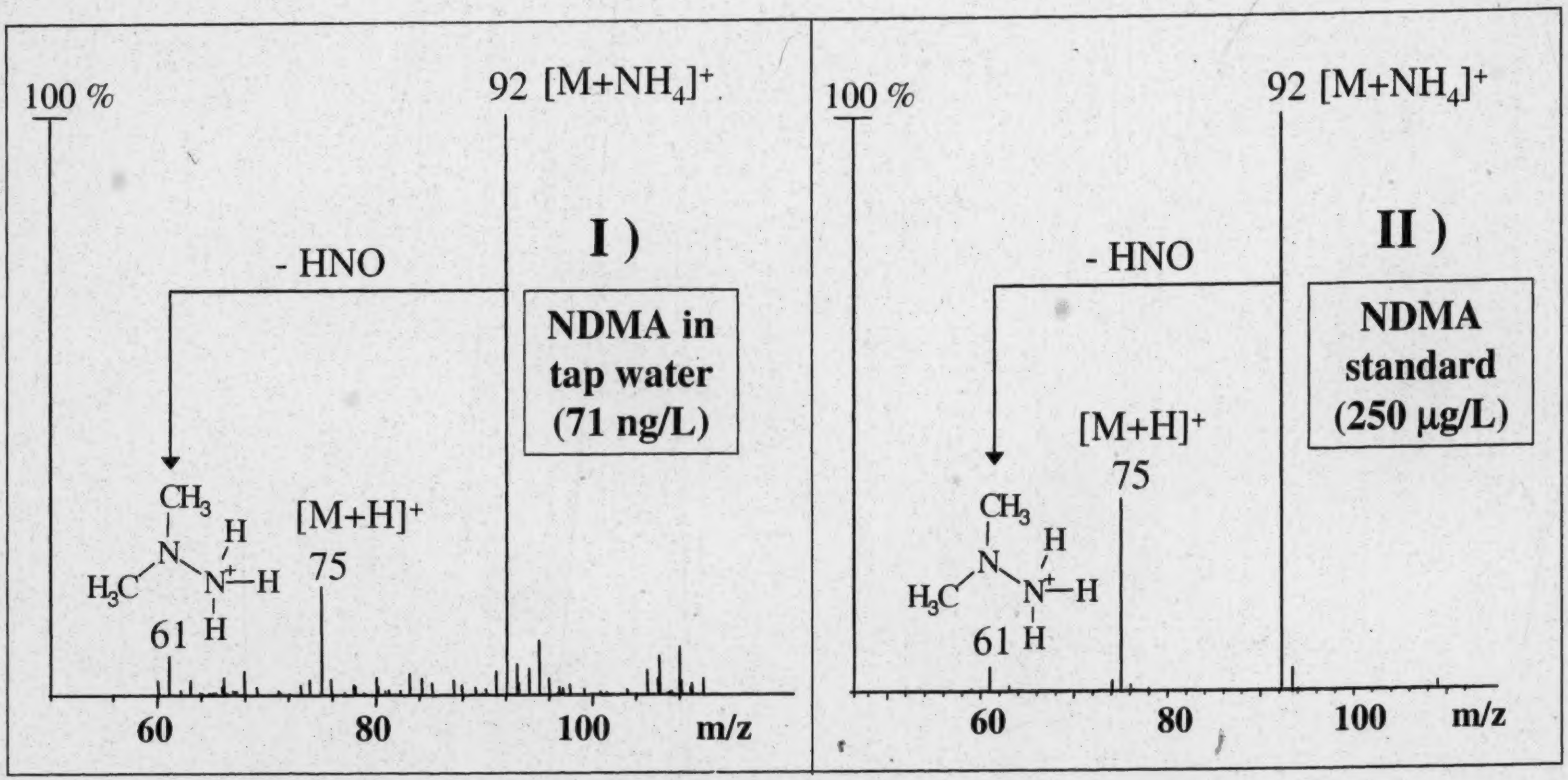

Figure 2.3. Full scan, ammonia positive chemical ionization, spectral comparison of *N*-nitrosodimethylamine (NDMA) from I) an authentic drinking water sample and II) an NDMA stock-standard solution.

Average absolute recoveries (%), using IS, were also used to assess method performance for the eight spiked *N*-nitrosamines and NDMA-*d6* **(Table 2.3)**. The average absolute recoveries from spiked water samples (10–200 ng/L; n=5) ranged from 78% for NPip to 110% for NDMA-*d6*. The RSDs for the average recoveries ranged from 6% to 13%. A good linear fit (R^2 near 1.0) and consistent recoveries for all *N*-nitrosamine species indicated NDMA-*d6* was a suitable surrogate for the other *N*-nitrosamines of interest when using the ID/SS procedure for quantification.

Methods for NDMA that use only Ambersorb® 572 report much lower recoveries (20%–40%) compared to the extraction technique reported here (Taguchi et al., 1994; Jenkins et al., 1995). Increased recoveries for our method may be attributed to the use of LiChrolut®EN in combination with the Ambersorb® 572. LiChrolut®EN has a smaller particle size distribution range than does Ambersorb® 572. Packing LiChrolut®EN on the bottom results in lower flow rates versus Ambersorb® 572 only. Reduced flow rates through the SPE bed allowed for better sample contact and increased sorption of analytes onto the SPE phases, compared to other extraction methods.

Isotope dilution methods rely on the *N*-nitroso species / NDMA-*d6* ratio and are independent of analyte recoveries, which was why ID was the early method of choice for NDMA quantification (Taguchi et al., 1994). Isotopically labeled standards, however, are not currently available for all *N*-nitrosamines of interest. With our SPE method, we were able to achieve much higher recoveries for eight *N*-nitrosamines and were therefore able to use an IS procedure for quantification.

Besides measuring recoveries for eight *N*-nitrosamines in calibration-standard solutions, use of an IS method also allowed for NDMA-*d6* recoveries to be determined. Having NDMA-*d6* recovery data from the IS method provided additional information about extraction efficiencies for authentic drinking water samples.

Table 2.2. Mass spectrometry: *N*-nitrosamine species performance using ammonia positive chemical ionization.

Compound[a]	Qualifier / Target Ratio[b] (RSD[c] %) (n=7)	Average RRF[d] (RSD%) (n=8)	R^2 (NDMA-*d6*)[e] (n=5)
NDMA	0.23 (5)	1.4 (7)	0.9992
NDMA-*d6*	0.18 (4)	1.4 (9)	-
MENA	0.32 (4)	1.7 (9)	0.9996
DENA	0.42 (2)	1.5 (5)	0.9992
DPNA-*d14*	0.55 (1)		-
DPNA	0.49 (2)	1.0 (6)	0.9994
NMor	0.24 (2)	0.8 (3)	0.9993
NPyr	0.48 (1)	0.8 (11)	0.9991
NPip	0.48 (1)	1.0 (6)	0.9992
DBNA	0.61 (1)	0.5 (5)	0.9960

[a] See **Table 2.1** for abbreviation descriptions.

[b] See **Table 2.1** for target- and qualifier-ion descriptions.

[c] Relative standard deviation (RSD).

[d] Relative response factor (RRF), calculated using DPNA-*d14* as internal standard.

[e] R^2 as linear response from *N*-nitrosamine species calibration curves using NDMA-*d6* as surrogate standard. Calibration curves (linear responses) were calculated by individually plotting *N*-nitroso species target-ion area ratios / NDMA-*d6* target-ion area ratios versus known *N*-nitroso species concentrations from extracted calibration-standard solutions (ng/L).

Table 2.3. Performance of isotope dilution / surrogate standard (ID/SS) and internal standard (IS) procedures for a range of *N*-nitrosamine concentrations[a].

Compound[b]	ID/SS Procedure		IS Procedure	
	Average Accuracy (%) (RSD[c] %)	Range (%)	Average Absolute Recovery (%) (RSD%)	Range (%)
NDMA-*d6*	NA[d]	NA	110 (13)	87–124
NDMA	100 (7)	93–111	98 (6)	87–102
MENA	97 (7)	87–105	94 (8)	80–99
DENA	98 (4)	95–104	91 (11)	76–101
DPNA	101 (2)	100–105	83 (12)	66–91
NMor	97 (5)	92–104	87 (12)	69–94
NPyr	94 (9)	82–103	84 (11)	67–91
NPip	102 (4)	99–107	78 (9)	66–82
DBNA	100 (9)	89–111	91 (10)	78–105

[a] *N*-nitrosamines were spiked into reagent water producing a range of concentrations (10-200 ng/L; n=5). NDMA-*d6* was spiked at 50 ng/L for all samples. Spiked reagent water was solid-phase extracted, analyzed by GC/MS ammonia positive chemical ionization and quantified using ID/SS and IS procedures.

[b] See **Table 2.1** for abbreviation descriptions.

[c] Relative standard deviation (RSD).

[d] NA = not applicable, NDMA-*d6* was used as the surrogate standard for ID/SS.

2.3.4 Method detection limits (MDL)

To evaluate the sensitivity of the instrument, 1.8 pg of *N*-nitrosamine stock-standard solution (1 μL at 1.8 pg/μL) was injected. This resulted in average signal-to-noise (S/N) ratios for the 92 and 75 m/z ions of approximately 17 and 4 respectively.

To evaluate method detection limits (MDLs) for each *N*-nitrosamine species in ammonia PCI mode, calibration-standard solutions (9.4 ng/L) were made in triplicate and extracted. Estimated MDLs were calculated as 3 × standard deviation of three independently spiked samples. Based on our spiking experiments, the estimated MDLs, using the ID/SS method, for the eight *N*-nitrosamines tested ranged from 0.4 ng/L for NPyr to 1.6 ng/L for NDMA and NPip (**Table 2.4**). Method detection limits were comparable using either ID/SS or IS quantification, however, the range of MDLs using the IS method was slightly lower. For tested *N*-nitrosamines, using the IS method, MDLs ranged from 0.4 ng/L for MENA to 1.3 ng/L for NPip and DBNA.

2.3.5 *N*-Nitrosamine analytical round-robin: an opportunity for method validation

Fourteen laboratories representing commercial, utility, regulatory and academic sectors (including the University of Alberta's Environmental Health Sciences Laboratory), were invited to take part in an international, blinded, analytical round-robin, which was part of a larger project sponsored by the WateReuse Foundation (WRF; Arlington, VA) entitled "*Alternative Methods for the Analysis of NDMA and Other Nitrosamines in Water and Wastewater*". The primary objective the WRF project was, "to develop alternative and reproducible analytical techniques for measuring NDMA and other nitrosamines in various matrices, including wastewater, recycled water, surface water and groundwater samples". For a more complete description of the analytical round-robin, please see **Appendix A**. **Table 2.5** highlights the excellent performance of the analytical method developed in this thesis.

Table 2.4. Calculation of method detection limits (MDL) for eight *N*-nitrosamine species.

Compound[a]	Average S/N[b] ± Std Dev	Isotope Dilution / Surrogate Standard			Internal Standard		
		Concentration (ng/L) (RSD[c] %)	Std. Dev.	Estimated MDL[d] (ng/L)	Concentration (ng/L) (RSD%)	Std. Dev.	Estimated MDL[d] (ng/L)
NDMA	29 ± 2	8.8 (6)	0.5	1.6	9.4 (2)	0.2	0.7
MENA	16 ± 4	8.2 (5)	0.4	1.2	8.9 (2)	0.1	0.4
DENA	41 ± 16	8.9 (5)	0.4	1.3	9.6 (2)	0.2	0.6
DPNA	134 ± 15	9.5 (2)	0.2	0.7	8.6 (4)	0.3	1.0
NMor	33 ± 7	8.7 (3)	0.2	0.7	8.9 (4)	0.3	1.0
NPyr	33 ± 11	7.7 (2)	0.1	0.4	8.1 (5)	0.4	1.2
NPip	37 ± 10	9.4 (6)	0.5	1.6	7.8 (6)	0.4	1.3
DBNA	41 ± 10	8.4 (4)	0.3	1.0	8.3 (5)	0.4	1.3

[a] See **Table 2.1** for abbreviation descriptions.

[b] Signal-to-noise ratio (S/N) for target-ion.

[c] Relative standard deviation (RSD).

Table 2.5. ***N*-Nitrosamine comparison of analytical round-robin samples between University of Alberta and Gold Standard (GS) Laboratories.**

Sample[a]	Compound[b]	Lab: U of A	Lab: GS1[c]	Lab: GS2[d]	UA/Mean (GS's)
		Concentration (ng/L)			(% Difference)
RR-01	NDMA	4.1	4.5	4.2	-5
	NMor	0.7	<2	<1	NA
RR-02	NDMA	17	16	17	0.3
	MENA	13	10	11	27
	DENA	10	12	12	11
	DPNA	12	14	11	-4
	NMor	17	19	16	-4
	NPyr	13	12	12	6
	NPip	11	10	11	4
	DBNA	13	12	12	7
RR-03	NDMA	14	14	14	0.6
	NMor	3.0	<2	2.3	29
RR-04	NDMA	160	170	170	-4
RR-05	NDMA	540	600	620	-12
	MENA	380	440	350	-5
	DENA	360	310	330	14
	DPNA	340	380	370	-11
	NMor	350	400	420	-14
	NPyr	370	420	400	-10
	NPip	340	400	370	-12
	DBNA	350	310	350	4.7
RR-06	NDMA	760	860	840	-10

[a] See **Appendix A** for full details on sample descriptions.

[b] See **Table 2.1** for abbreviation descriptions.

[c] Gold Standard Laboratory 1 (GS1).

[d] Gold Standard Laboratory 2. (GS2).

NA = not applicable.

Note: % differences may not exactly match due to rounding, please see Appendix A for raw data.

2.3.6 *N*-Nitrosamines in authentic drinking water samples

Drinking water samples were collected in July and September 2003 from City A and Town B in Alberta, Canada. City A is downstream of Town B, and they both use surface-water sources. The two locations are approximately 40 km apart. City A uses chloramination in combination with UV, while Town B uses only chloramination for disinfection. Samples were collected within the drinking water distribution systems of both locations. Finished water samples (water treatment plant (WTP) effluent) were only collected in City A. During the July sampling, *N*-nitrosamines were not detected in City A's source water. After treatment, the NDMA concentration in City A's finished water was 67 ng/L, which increased to 180 ng/L within the distribution system (**Table 2.6**). It is important to point out that the high NDMA concentrations in City A's water supply were measured from water being distributed to consumers following normal treatment plant practices. *N*-Nitrosamine concentrations did not result from artificially harsh experimental conditions. The concentration of NDMA in City A's drinking water was much higher compared to those reported in a 2001-2002 North American survey, which found median NDMA concentrations within selected chloraminated distribution systems of < 2 ng/L (Barrett et al., 2003). Barrett et al. (2003) observed generally increasing NDMA concentrations within the distribution system compared to WTP effluent samples, as was also the case with City A. Distribution system concentrations in City A exceeded California's Action Level of 10 ng/L (CDHS, 2004) and Ontario's interim maximum acceptable concentration (IMAC) of 9 ng/L (OMOE, 2004) by up to 20-fold. *N*-Nitrosopyrrolidine was also detected in City A's distribution system (4 ng/L) but not in samples collected at the treatment plant. In Town B, distribution system NDMA concentrations were 4 ng/L. No other *N*-nitrosamine species were detected in Town B's distribution system. Sub-samples (July) from City A and City B were sent to an independent lab for confirmatory analysis using GC/HRMS (EI) with quantification by ID (Guo et al., 2001). Although the number of samples sent for validation was limited, there was good agreement between our ammonia PCI method and the HRMS results (**Table 2.6**).

In September 2003 a second round of sampling was conducted, which included more distribution samples from City A. Overall, the NDMA concentrations for City A in September were reduced to 21%–32% of the July values at the same sampling locations.

Sample D1 was collected at an intermediate distribution location, while samples D2–4 were remote locations from throughout City A's distribution system. Samples collected at locations D2–4 were chosen because they represented locations with maximum water residence times for City A. An increase in NDMA concentrations was seen when comparing distribution system samples to finished water samples at the WTP. For the September sampling in City A, NDMA concentrations appeared generally to increase with increasing residence times. Location D4 was the furthest point examined in the system and it had the highest NDMA concentration (81 ng/L) (**Table 2.7**) for that sampling period. The concentration of NPyr was 2 ng/L within the remote parts of the distribution system. A third *N*-nitrosamine, NMor, was detected at 1 ng/L in City A's distribution system at location D3. The samples collected at the WTP for City A did not show detectable concentrations of either NMor or NPyr.

The September distribution system concentration of NDMA in Town B was reduced (50%) compared to July. During the September sampling, Town B's distribution system NDMA concentration (2 ng/L) was again much lower compared to City A (14–81 ng/L). No other *N*-nitrosamine species were detected in Town B's distribution system.

During the July and September samplings, all trip blanks were free of detectable *N*-nitrosamines. Surrogate recoveries for NDMA-*d6* ranged from 60% to 114% for the two sampling events. The lowest recovery (60%) was from City A source water with high DOC (16 mg/L), which may partly explain the lower NDMA-*d6* recovery. In addition to drinking water, our method has achieved accurate results, within 10% of reference values, detecting a range of *N*-nitrosamine species at concentration levels up to 800 ng/L in challenging matrices such as secondary and tertiary wastewater effluent.

Detection of NPyr and NMor in distribution samples from City A represents the first time *N*-nitrosamines other than NDMA have been reported in drinking water. Other non-NDMA *N*-nitrosamines such as NPyr, NPip and *N*-Nitrosodiethylamine (DENA) have been reported in wastewater before and after chlorination (Mitch and Sedlak, 2002b). The presence of *N*-nitrosamines, other than NDMA, raises questions about the origin and formation potentials of *N*-nitrosamines in drinking water. NDMA can form from amine-based coagulants such as diallyldimethylammonium chloride (DADMAC) at WTPs conducting chloramination (Wilczak et al., 2003) as well as in jar tests using either DADMAC or epichlorohydrin-dimethylamine (Epi-DMA) under chlorination conditions

Table 2.6. Detection of *N*-nitrosamines in source, finished and distribution water samples (July 2003). Application and validation of the solid-phase extraction GC/MS ammonia positive chemical ionization (PCI) method using isotope dilution / surrogate standard (ID/SS) and internal standard (IS) procedures.

Sample Location	GC/MS (Ammonia PCI)					GC/HRMS[a] (EI[b])		DOC[c] Concentration (mg/L)
	Concentration (ng/L)				NDMA-*d6*[d] Recovery (%) (RSD[e] %)	Concentration (ng/L)		
	NDMA[f] (RSD%)		NPyr[f] (RSD%)		NDMA-*d6*[f]	NDMA	NPyr	
	ID/SS	IS	ID/SS	IS				
City A Source	ND	ND	ND	ND	60	ND	N/A	16
City A Finished	71	67	ND	ND	104	84	N/A	9
City A Distribution 1	180 (15)	180 (15)	4 (7)	4 (4)	114 (11)	160	2	9
Town B Distribution	5 (4)	4 (5)	ND	ND	97 (11)	6	N/A	9

[a] Analysis of split-samples conducted by Metropolitan Water District of Southern California (MWDSC). One-liter sample bottles were shipped, via an overnight courier, to MWDSC in coolers with frozen blue-ice packs for GC/HRMS analysis. Samples were tracked and received the following morning. Samples analyzed by MWDSC were extracted and analyzed according to a method described previously (Guo et al., 2001).

[b] Electron ionization (EI).

[c] Dissolved organic carbon (DOC); determined by combustion oxidation (680 °C) with non-dispersive infrared detection.

[d] NDMA-*d6* was used as a surrogate standard.

[e] Relative standard deviation (RSD).

[f] See **Table 2.1** for abbreviation descriptions.

ND = not detected.

N/A = not analyzed.

Note: Trip blanks analyzed by UA and MWDSC were ND for all *N*-nitrosamines.

Table 2.7. **Detection of *N*-nitrosamines in finished and distribution drinking water samples (September 2003) using solid-phase extraction GC/MS ammonia positive chemical ionization (PCI) with internal standard (IS) quantification.**

Sample Location	Concentration (ng/L)			NDMA-*d6*[a] Recovery (%)
	NDMA[b]	NMor[b]	NPyr[b]	
City A Finished	14	ND	ND	67
City A Distribution[c] 1	57	ND	ND	109
City A Distribution[c] 2	73	ND	2	85
City A Distribution[c] 3	63	1	2	70
City A Distribution[c] 4	81	ND	2	83
Town B Distribution	2	ND	ND	73

[a] NDMA-*d6* was used as a surrogate standard.

[b] See **Table 2.1** for abbreviation descriptions.

[c] Distribution locations for City A are listed in order of increasing distance from the water treatment plant.

ND = not detected.

(Kohut et al., 2003). Both DADMAC and EPI-DMA contain dimethylamine, a known precursor of NDMA (Mitch and Sedlak, 2002a). Formation of NPyr and NMor suggests a research need for other sources of *N*-nitrosamine precursors as well as alternative formation mechanisms.

More data are required from additional utilities to better understand the occurrence and formation of NDMA as well as other *N*-nitrosamines in drinking water. Research is partially limited because of costly, highly labour intensive and low throughput analytical methods. Our dual media SPE method has the potential to be automated using commercial SPE cartridges and manifolds, which would dramatically increase sample throughput and reduce overall costs.

The analytical internal standard method described herein has the potential to characterize a variety of hydrophilic *N*-nitroso compounds in water, which may have health risk implications. We foresee application of this SPE GC/MS ammonia PCI method as an important advancement for exploring emerging disinfection by-products.

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Chapter Three

Occurrence of *N*-Nitrosamines in Alberta Public Drinking-Water Distribution Systems[1]

3.1 Introduction

The existence of trace concentrations (μg/L) of halogenated disinfection by-products (DBPs) in drinking water, such as trihalomethanes (THMs), has been known for over 30 years (Rook, 1974; Bellar et al., 1974). However, public health concerns surrounding DBPs persist, despite large efforts and millions of dollars being spent on investigating adverse health outcomes and engineering process-control research. Simply stated, DBPs result from chemical reactions between precursors such as natural organic matter in source water and disinfectants such as chlorine, chloramines, or ozone. A 1976 report published by the National Cancer Institute identified chloroform, typically the most abundant THM in drinking water, as a carcinogen in laboratory animals (NCI, 1976). Around the same time, results from national drinking water surveys indicated that THMs were ubiquitous in chlorinated drinking water throughout the United States (Symons, 1975; USEPA, 1978). Exposure to THMs through drinking water combined with chloroform being deemed a carcinogen sparked an ongoing public health debate and fueled a new era of safe drinking water regulations and research initiatives.

Managing risks for the drinking water industry requires balancing: i) immediate and certain microbial pathogen risks and ii) delayed and uncertain hazards resulting from exposures to DBPs. Failure to disinfect adequately clearly leads to conditions that may have catastrophic outcomes, as the fatal Cabool, MO (1990), Gideon, MO (1993), Washington County, NY (1999), and Walkerton, ON (2000) outbreaks remind us (Hrudey and Hrudey, 2004). Thus the ultimate priority for any drinking water provider must be pathogenic microbial control. Once immediate human health risks are controlled, however, a precautionary approach dictates an evaluation of more uncertain risks, such as adverse outcomes from exposures to DBPs.

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[1] A version of this chapter has been submitted (June 2005) for publication. Charrois, J.W.A., Boyd, J.M., Froese, K.L., and Hrudey, S.E. *Journal of Environmental Engineering and Science.*

An assessment of health risks requires an understanding of the magnitude and frequency of DBP exposures. Numerous national drinking water surveys, including but not limited to those in the United States (Krasner et al., 1989; Arora et al., 1997), Canada (Health Canada, 1995, 1996; Conrad, 1997; Charrois et al., 2004*a*), and Australia (Simpson et al., 1998) have provided DBP occurrence data. To date, hundreds of DBP species representing a variety of chemical classes, which are produced under a range of disinfection conditions in a variety of source waters have been identified (Krasner et al., 1989; Richardson, 1998). Regulatory actions have almost exclusively been directed towards halogenated DBPs, primarily THMs and haloacetic acids (HAAs). Historically, the focus on halogenated DBPs was partly due to the limited availability of analytical techniques to detect these groups of compounds as well as their relatively high abundance in drinking waters. Unfortunately, an over-reliance on historical analytical characterization led regulators, researchers and the water industry to a preoccupation with halogenated DBPs. Even in a recent occurrence study of "high priority" DBPs, the majority of compounds (91%; 59 of 65 DBPs) included in the study were still halogenated (Weinberg et al., 2002). As instrumentation and analytical methods improve, so have abilities to identify and quantify new compounds in drinking water. While there is a natural expectation that chlorine will produce halogenated DBPs, other non-halogenated DBPs are commonly produced as well (Froese et al., 1999). Too often health concerns have been attributed to the mere detection of a compound. As method detection limits are continually lowered, more compounds are inevitably detected. This trend demands that health significance must be judged on more than just detection at an arbitrary method detection limit.

In epidemiology studies, urinary bladder cancer is the cancer associated most consistently with lifetime exposures to disinfected drinking water. Like monitoring efforts, much of the adverse health-effects research has also focused on exposures to THMs and HAAs. A Health Canada Expert Working Group concluded that it was "possible" to "probable" that chlorinated by-products pose a significant risk to the development of cancer, particularly bladder cancer (Mills et al., 1998). Yet the same consensus report found that the identified halogenated by-products could not explain the relative risks estimated from epidemiology studies. Another expert panel assembled by Health Canada in 2002 agreed that total THMs can be a surrogate for exposure to chlorinated DBPs in epidemiology studies, however, the "...total THM level is often driven by chloroform, the predominant THM, and toxicological evidence to date does not support a causal relationship between chloroform and adverse health outcomes at currently regulated drinking water exposure levels" (SENES, 2003; pp. S2). Thus, while epidemiology studies have found low but consistent associations between increased consumption of disinfected drinking water and chronic adverse outcomes such as urinary bladder cancer, plausible agents are yet to be identified. Even if the risks of adverse health outcomes from exposures to disinfected drinking water are low, the population attributable risk could potentially still be significant, therefore well-focused DBP research is still necessary.

N-nitrosamines are an emerging class of DBPs, which are 2 to 4 orders of magnitude more potent than regulated halogenated DBP species, specifically THMs and HAAs. The United States Environmental Protection Agency's (USEPA) weight-of-evidence characterization for chloroform, a regulated THM and often the most abundant DBP species, concludes that chloroform is likely to be carcinogenic under high exposure conditions that lead to cytotoxicity and regenerative hyperplasia (USEPA, 2001). Accordingly, at relatively low exposure concentrations, such as those typical in drinking water, chloroform is not carcinogenic.

Interest in *N*-nitrosodimethylamine (NDMA) as a DBP began in 1989 after its discovery in Ohsweken, Ontario drinking water (Jobb et al., 1994). In 1994 NDMA was added to a list of parameters included in Ontario's Drinking Water Surveillance Program (DWSP). Today NDMA remains part of the DWSP (OMOE, 2004*a*). In 1998, NDMA was identified in drinking waters in California (CDHS, 2005*a*), which initiated several new research programs. Typical NDMA concentrations reported in drinking water are in the sub-ng/L to 10 ng/L range, whereas wastewater concentrations are in the hundreds of ng/L range or more (Najm et al., 2001). However, in at least one Canadian location, NDMA drinking water concentrations of up to 180 ng/L were recently reported (Charrois et al., 2004*b*). The key data gap for NDMA risk assessments is a lack of occurrence data in public water distribution systems (USEPA, 2005). If exposures to *N*-nitrosamines in drinking water are shown to be a human health risk, they will need to be minimized wherever they occur at unacceptably high levels.

Research on NDMA indicates it can form in drinking water and wastewater after chlorination (Mitch et al., 2002) or chloramination (Najm et al., 2001; Choi et al., 2002). The USEPA categorizes NDMA as a "probable" human carcinogen (class B2) based on multiple site tumor induction in rodent and nonrodent mammals exposed by various routes (USEPA, 1987). Relatively speaking, NDMA is more toxicologically potent (Ashbolt, 2004) compared to "traditional" DBPs such as THMs and HAAs.

Currently, there are no federal drinking water standards for NDMA in Canada or the United States. While no national NDMA research initiatives are currently underway in Canada, the USEPA has already developed Method 521 for the detection of seven *N*-nitrosamines in drinking water (USEPA, 2004). It is anticipated that Method 521 would support the proposed national monitoring of *N*-nitrosamines resulting from inclusion in the Unregulated Contaminant Monitoring Rule (UCMR), thus establishing national occurrence and frequency data and ultimately supporting a federal regulatory determination if necessary. Two jurisdictions in North America have developed their own NDMA guideline values for drinking water, Ontario's Drinking-Water Quality Standard (ODWQS) is 9 ng/L (OMOE, 2003) and California's Notification Level (NL) is 10 ng/L (CDHS, 2005*b*). As of May 2005, California also established NLs for *N*-nitrosodiethylamine and *N*-nitrosodi-*N*-propylamine of 10 ng/L each. The USEPA, through the Integrated Risk Information System (IRIS) database, established a 1×10^{-6} upper-bound lifetime cancer risk for NDMA in drinking water at 0.7 ng/L (USEPA, 1987). Additional drinking water risk-based values are available through the IRIS database for: *N*-nitrosodi-*N*-butylamine, *N*-nitroso-*N*-methylethylamine, *N*-nitrosodi-*N*-propylamine, *N*-nitrosodiethanolamine, *N*-nitrosodiethylamine, *N*-nitrosodiphenylamine, and *N*-nitrosopyrrolidine.

After 30 years of halogenated DBP research, chemical water-quality priorities in the drinking water industry need to be expanded to include more that than just halogenated compounds. In 2001 Dr. Richard Bull called for a concerted effort to investigate conditions that generate NDMA and other potential *N*-nitrosamine DBPs (Bull, 2001). We propose a refocusing of drinking water research priorities in Canada to include alternative DBP classes, such as *N*-nitrosamines, which are more toxicologically potent compared to THMs and HAAs (Bull, 2001). The main objectives of this study were to: (1) develop an efficient solid-phase extraction (SPE) method for *N*-nitrosamines, which facilitates rapid processing of multiple samples, and (2) evaluate select public drinking-water distribution systems in Alberta for the occurrence of eight *N*-nitrosamine species.

3.2 Materials and Methods

3.2.1 Reagents and standards

Methanol (AnalaR®) and dichloromethane (Omni-Solv®) were purchased from VWR Canlab (Mississauga, ON). Hexane and reagent water (Optima Grade) as well as sodium bicarbonate (ACS reagent grade) were obtained from Fisher Scientific (Nepean, ON). Chlorine solutions were prepared by diluting purified grade (4%–6%) NaOCl (Fisher Scientific) into reagent water. The L-ascorbic acid (min 99.0%) was obtained from Sigma-Aldrich (Oakville, ON). Solid-phase extraction (SPE) materials, Ambersorb® 572 (Rohm & Haas; Philadelphia, PA) and LiChrolut® EN (Merck; Darmstadt, Germany) were supplied through Supelco (Oakville, ON) and VWR Canlab respectively. A standard solution containing nine *N*-nitrosamines (*N*-nitrosodimethylamine, NDMA; *N*-nitrosomethylethylamine, MENA; *N*-nitrosodiethylamine, DENA; *N*-nitrosodi-*N*-propylamine, DPNA; *N*-nitrosomorpholine, NMor; *N*-nitrosopyrrolidine, NPyr; *N*-nitrosopiperidine, NPip; *N*-nitrosodi-*N*-butylamine, DBNA; and *N*-nitrosodiphenylamine, DPhNA) was purchased from Supelco. Isotopically labeled standards, (98%) ([6-^{2}H] *N*-nitrosodimethylamine, NDMA-*d6* and [14-^{2}H] *N*-nitrosodi-*N*-propylamine, DPNA-*d14*) were from Cambridge Isotope Laboratories (Andover, MA). *N*-Nitrosamines are suspected human carcinogens, all necessary health and safety precautions should be taken when handling samples containing or suspected of containing these compounds.

3.2.2 Sample collection

Samples were collected in pre-cleaned amber glass bottles with PTFE lids. During sample collection, bottles were filled to zero headspace and 20 mg/L of powdered L-ascorbic acid (sample preservative) was added. Samples were immediately placed into coolers with icepacks and transported to the laboratory, where they were refrigerated at 4 °C until extraction, typically within 1 week. Field blanks, consisting of reagent water with 20 mg/L of L-ascorbic acid, were included with each sampling trip and handled like authentic samples, except the bottles were not opened in the field.

All utilities surveyed used either chlorination- or chloramination-based disinfection processes. The water treatment plants selected served a majority of Alberta's population. Inclusion criteria for facility selection included: a history of elevated THMs or HAAs (Conrad, 1997) and/or naturally high ammonia concentrations in groundwater sources.

3.2.3 Sample preparation and analysis of *N*-nitrosamines

Authentic drinking water samples were extracted for analytes of interest using a dual media, off-line SPE procedure. A 12-port Visiprep™ DL SPE vacuum manifold with a glass collection tank (Supelco) containing a customized PTFE extraction-tube rack was used in an attempt to increase sample throughput, compared to our previously described method (Charrois et al., 2004*b*). The customized extraction-tube rack held 10 X 50 mL glass extraction tubes. The extraction tubes were from a TurboVap®II Concentration Workstation (Zymark Corporation (now Caliper Life Sciences); Hopkinton, MA). The manifold cover houses flow control valve stems that can be individually adjusted to vary the flow of each sample during extraction. Passing through each control valve was a disposable liner, consisting of a polypropylene luer hub attached to a thin-walled PTFE tube. Reusable glass SPE cartridges (6 mL) with glass frits containing: 350 mg LiChrolut® EN (bottom), 500 mg Ambersorb® 572 (middle), and a wad of glass wool (top) fit into the luer hubs. The SPE bed was washed with hexane and then dichloromethane, followed by conditioning with methanol, as previously described (Charrois et al., 2004*b*).

A surrogate standard (SS), NDMA-*d6* (20 ng), was spiked into each 500 mL eluent (*i.e.* authentic drinking water sample, laboratory fortified blank, or laboratory reagent blank). Up to 10 samples could be simultaneously extracted using the manifold system. Under slight vacuum, each sample was continuously transferred through an individual PTFE transfer line (3.2 mm; 1/8 inch diameter) from a sample bottle, through the SPE cartridge, and into the glass collection tank. Each transfer line had a stainless steel weight on one end (sample bottle) and a PTFE adaptor plug, which fit into the top of the SPE tube. During extraction, water ponded on top of the SPE materials, before passing through. Flow rates were approximately 3–6 mL/min under slight vacuum.

When all extractions were complete, a full vacuum (-30 kPa) was applied, to dry the SPE materials. Elution of SPE beds followed using 20 mL of dichloromethane. Eluates were collected in 50 mL glass extraction tubes and concentrated using a TurboVap®II concentrator to a final volume of ca. 100 μL. The TurboVap®II used ultra-high-purity N_2 (60 kPa) and the water bath was set for 40 °C. An internal standard (IS), DPNA-*d14* (20 ng), was spiked into each concentrated eluate and the resulting solution was transferred into a conical glass insert inside a 2 mL amber glass GC vial. Extracts were stored at 4 °C until analysis.

N-Nitrosamines from sample extracts were chromatographically separated and quantitatively analyzed using an internal standard procedure with GC/MS ammonia positive chemical ionization (PCI), based on our previous method (Charrois et al., 2004*b*). Briefly, quantification by internal standard involved direct GC/MS ammonia PCI analysis of stock-standard solutions (5 to 350 pg/μL), which contained: nine *N*-nitrosamines as well as added DPNA-*d14* and NDMA-*d6*. Area responses were used to calculate relative response factors (RRFs) for each compound. The internal standard (DPNA-*d14*) was used to determine RRFs for NDMA-*d6* and for the analytes of interest. Relative standard deviations (RSDs) for each RRF, over the range of concentrations tested, were ≤ 10%, so an average RRF was calculated for each *N*-nitrosamine species and used for quantification.

3.2.4 Quality assurance and quality control

For quality assurance purposes, an extra sample was collected at most sampling sites, and sent to one of two third-party laboratories for independent *N*-nitrosamine analysis. The first independent laboratory (IL1) used a continuous liquid-liquid extraction technique coupled with GC/MS ammonia PCI to analyze for: NDMA, MENA, DENA, DPNA, NPyr, NPip, and DBNA (Eaton, 2004). The second independent laboratory (IL2) used a solid-phase extraction procedure combined with GC high-resolution mass spectrometry for quantification of: NDMA, DENA, NMor and DBNA (OMOE, 2004*b*, 2004*c*). Authentic drinking water samples in our lab were extracted and analyzed in triplicate, unless otherwise indicated. In addition to authentic drinking water samples, each sample batch included an extracted field blank or laboratory reagent-water blank and a laboratory fortified blank (LFB).

All glassware, including SPE extraction tubes were washed, solvent rinsed, and baked at 370 °C overnight. Sample bottles were cleaned in a similar manner and stored overnight at 170 °C. The PTFE transfer lines were cleaned by first rinsing with 500 mL of a 1:1 (v/v) methanol : reagent water solution, followed by 500 mL hexane, and finally 500 mL of reagent water.

3.2.5 Combined chlorine residual

Samples for combined chlorine residual analysis were collected in pre-cleaned amber glass bottles. Sample bottles were treated overnight by adding 1 mL of NaOCl (~5%) to 1000 mL of reagent water, to remove chlorine demand. Bottles were rinsed with reagent water, baked in the oven at 170 °C overnight, and triple rinsed with sample water, prior to use. Combined chlorine residual concentrations were determined using amperometric forward titration procedures based on *Standard Methods* (APHA, 1998). Automated titrations were made using the AutoCAT™ 9000 (Hach Company; Loveland, CO). Chlorine reference solutions, obtained from the Hach Company, were diluted and analyzed to ensure accurate method performance.

3.2.6 Routine water quality

Samples for routine water quality parameters were collected and handled under the same conditions as those samples for *N*-nitrosamine and chlorine residual analysis. After collection, samples were delivered to a commercial laboratory for processing. Routine water quality parameters (pH, DOC, UV_{254}, NO_3^-, NH_3-N, and total Kjeldahl nitrogen (TKN)) were analyzed according to *Standard Methods* (APHA, 1998). Organic nitrogen was calculated as the difference between TKN and NH_3-N.

3.3 Results and Discussion

3.3.1 Overview

Results suggest successful incorporation of a commercially available SPE manifold, providing increased sample throughput (up to 10 samples per day), while maintaining analytical performance parameters such as relevant detection limits (5 ng/L for NDMA) and high average recoveries of the surrogate standard (66%–100%) within authentic drinking water samples. Additionally, our one-time survey of 20 Alberta distribution systems identified six locations (30%) with detectable NDMA concentrations. Two of the six locations contained NDMA above the ODWQS of 9 ng/L.

Overall, distribution systems from five of twelve (42%) chloraminating utilities had detectable NDMA concentrations ranging from 1.3 ng/L (detected by an independent laboratory) to 100 ng/L. Chloraminating facilities included both those utilities that routinely practiced chloramination (n = 8) as well as locations that chlorinated groundwater with naturally high ammonia concentrations (n = 4). Considering all utilities, 14 of 20 (70%) distribution systems had NDMA concentrations < 5 ng/L. Finally, two additional *N*-nitrosamine species, *N*-nitrosomorpholine (NMor) and *N*-nitrosopyrrolidine (NPyr), were quantified in two chloraminating distribution systems, highlighting the importance of analyzing for multiple *N*-nitrosamine species, not only NDMA.

3.3.2 Quality assurance and quality control

A total of seven field blanks and two laboratory reagent-water blanks were analyzed. *N*-Nitrosamine species were not detected in blank samples, with the exception of NDMA. The presence of a low background of NDMA or NDMA-like species appeared in all blanks with an average calculated concentration of 2.3 ng/L (SD = 0.9 ng/L). The range of calculated NDMA concentrations in the blank samples was from 0.9 ng/L to 3.7 ng/L. The two independent laboratories each analyzed one field blank, of identical composition to the ones in our laboratory. The first independent laboratory (IL1) reported all measured *N*-nitrosamine species as below their method-reporting limit (MRL) of 2 ng/L. The second independent laboratory (IL2) reported all measured *N*-nitrosamine species, except NMor, as below their reporting detection limit (RDL) of 1 ng/L, while NMor was below their RDL of 2 ng/L.

Low-level background NDMA appeared to be coming from sources within our lab. We subsequently identified some brands of methanol, used to condition the SPE materials, as containing interfering compounds that chromatographically elute around the same time as NDMA.

3.3.3 Detection limits

Initially, detection limits for our method were calculated based on the analysis of seven independently prepared LFBs (5.2 ng/L). The standard deviation of the seven replicates was then used to calculate an $\mathrm{MDL}_{\mathrm{LFB}}$ for each compound (Glaser et al., 1981):

$$\mathrm{MDL}_{\mathrm{LFB}} = t_{(N\text{-}1,\ 1\text{-}\alpha=0.99)} \times \mathrm{S}_{\mathrm{LFB}} \qquad \textbf{(Equation 3.1)}$$

Where t is the one-tail Student t-test value for N-1 degrees of freedom, and S_{LFB} is the standard deviation of NDMA concentrations calculated from independent analysis of LFBs. Using the LFB approach the calculated $MDLs_{LFB}$, for NDMA, NMor, and NPyr (the detected *N*-nitrosamine species) were: 3 ng/L, 2 ng/L and 3 ng/L respectively. To address the low level background associated with NDMA, a more conservative determination based on an approach used by Martin et al. (2000) that incorporates calculated analyte concentrations measured in blanks, was used for determining the NDMA MDL_{Blk}:

$$MDL_{Blk} = \text{Average Blank Concentration} + (t_{(N-1,\ 1-\alpha=0.99)} \times S_{Blk}) \qquad \textbf{(Equation 3.2)}$$

Where t is the one-tail Student t-test value for N-1 degrees of freedom, and S_{Blk} is the standard deviation of NDMA concentrations calculated from independent analysis of extracted field blanks and laboratory reagent-water blank samples. Incorporating responses from the blank samples resulted in a more conservative MDL_{Blk} estimation of 5 ng/L for NDMA. There were no measurable signals for NMor or NPyr in the blanks so the MDL_{Blk} approach was not applied to those species.

3.3.4 Alberta *N*-nitrosamine survey

Distribution samples from select Alberta public drinking-water distribution systems (n = 20) were collected, extracted, and analyzed for eight *N*-nitrosamines during the summer of 2004 (**Table 3.1**). With the manifold system, up to ten samples per day could be extracted by one person. Increased sample throughput was a major improvement compared to our original SPE method, which could only process two samples per day. For a large-scale *N*-nitrosamine survey to be realized, extraction methods must have high throughput and not be prohibitively expensive to operate.

Source water composition among the survey sites was 75% surface water and 25% groundwater. In this study, 8 of 20 (40%) treatment plants used chloramination for disinfection while 8 of 20 (40%) used only chlorine. The remaining four treatment plants (20%) had source groundwaters with historically elevated, naturally occurring ammonia, which may have effectively resulted in a natural chloramination process. All routinely chloraminating utilities in Alberta were included in this survey except for one, which was excluded because of the small population the plant served and its general location relative to Edmonton. Efforts to include most of Alberta's chloraminating facilities allowed an examination of whether or not water treatment plants using chloramination were at a greater risk of generating NDMA compared to chlorination alone, as suggested in the literature (Najm et al., 2001; Wilczak et al., 2003).

A total of 23 locations from 20 public utilities were sampled between July and September, 2004. Of the samples collected, 6 of 20 (30%) distribution systems had at least one location where NDMA was detectable. Two of the locations, 'I' and 'L', had NDMA concentrations that were detected by IL2 but were reported by our laboratory as below the MDL_{Blk}. Of the utilities with detectable NDMA concentrations (as determined by our laboratory), 5 of 6 (83%) used chloramination, either as a chloraminating utility or as a utility that chlorinated groundwater with naturally elevated ammonia concentrations. Only one of eight (13%) chlorination-only utilities had detectable NDMA (12 ng/L). Detectable NDMA concentrations in authentic drinking water samples ranged from 1.3 ng/L (detected by IL2) to 100 ng/L. Seven of twelve (58%) chloraminated distribution systems had NDMA below our laboratory's MDL_{Blk} of 5 ng/L. Two distribution systems, Location 'A' (chloramination) and Location 'M' (chlorination), had NDMA concentrations above the ODWQS of 9 ng/L (OMOE, 2003). Distribution system 'A' had the highest NDMA concentrations, ranging from 66 ng/L at a location in the middle of the distribution system to 100 ng/L at the furthest extreme of the distribution system. Previous studies (Wilczak et al., 2003; Barrett et al., 2003) have also shown NDMA concentrations to increase with increasing retention time within distribution systems. Results from IL1 and IL2 helped to confirm the NDMA findings from our laboratory. Comparable NDMA results were obtained between our method and the methods used by IL1 and IL2 (**Table 3.1**). Slight differences in reported *N*-nitrosamine concentrations can be explained in part because of variations in the methods of extraction, analysis and quantification used amongst the three laboratories.

N-Nitrosamines other than NDMA were detected in 2 of 20 (10%) distribution systems (**Table 3.2**). *N*-Nitrosomorpholine (NMor) and *N*-nitrosopyrrolidine (NPyr) were detected at concentrations up to 3 ng/L and 4 ng/L respectively. In distribution system 'A', IL2 detected NMor at 2 ng/L and our laboratory detected NPyr at concentrations up to 4 ng/L, at extreme ends (high residence time) of the distribution system (locations A1 and A2). However, at a more central location (A3), neither NPyr nor NMor were detected. Thus residence time appears to be an important variable in the

Table 3.1. Quantification of *N*-nitrosodimethylamine (NDMA) detected in select chloraminated and chlorinated Alberta drinking-water distribution system samples (July–September 2004).

Location	Source Water	U of A[a]		IL1[b]	IL2[c]
		Concn (ng/L) ± RSD[d] (%)	NDMA-*d6*[e] Recovery (%)	Concn (ng/L)	Concn (ng/L)[f]
Chloramination					
A1	Lake	100 ± 9	73 ± 11	97	NA
A2	Lake	70 ± 13	66 ± 15	NA	100; 110
A3	Lake	66 ± 13	76 ± 14	75	NA
B	Creek	7.9; 8.1[f]	88 ± 1	NA	4.6; 4.3
C	River	6.0; 5.6[f]	83 ± 6	NA	4.8; 5.0
D	Lake	< 5	82 ± 7	< 2	NA
E	River	< 5	86 ±6	NA	< 1
F	River	< 5	83 ± 1	< 2	NA
G	River	< 5	72 ± 12	NA	NA
H1	River	< 5	81 ± 4	< 2	NA
H2	River	< 5	78 ± 11	NA	NA
Chlorinated groundwater containing naturally high ammonia					
I	Groundwater	< 5	80 ± 4	NA	4.0; 4.6
J	Groundwater	< 5	89 ± 4	NA	< 1
K	Groundwater	< 5	89 ± 3	NA	< 1
L	Groundwater	< 5	100 ± 2	NA	1.3; 2.7
Chlorination					
M	Groundwater	12 ± 4	92 ±4	17	13; 14
N	River	< 5	91 ± 7	NA	< 1
O	River	< 5	82 ± 5	NA	NA
P	River	< 5	68 ± 8	NA	NA
Q	River	< 5[f]	76 ± 8	NA	NA
R	Lake	< 5	79 ± 5	< 2	NA
S	Creek/River	< 5	80 ± 18	< 2	NA
T	Surface Runoff	< 5	83 ± 10	< 2	NA

[a] NDMA determined by solid-phase extraction and GC/MS ammonia positive chemical ionization with internal standard quantification; average concentration reported, n = 3 unless otherwise indicated.

[b] Independent laboratory one (IL1). NDMA determined by continuous liquid-liquid extraction with GC/MS ammonia positive chemical ionization with internal standard quantification (Eaton et al., 2004); single sample concentration reported.

[c] Independent laboratory two (IL2). NDMA determined by solid-phase extraction and GC/HRMS electron ionization with isotope dilution quantification (OMOE, 2004*b*); duplicate sample concentrations reported.

[d] Relative standard deviation.

[e] NDMA-*d6* used as a surrogate standard.

[f] Duplicate analysis; duplicate sample concentrations reported.

Note: NA, Not analyzed.

formation of NPyr and NMor, at least for conditions within the distribution system in Location 'A'. Distribution system 'A' results for NMor and NPyr remained consistent with earlier findings (Charrois et al. 2004*b*). A second, distribution system, location 'I' had a detectable NMor concentration of 3 ng/L that was confirmed by IL2. Neither IL1 nor IL2 analyzed for NPyr and thus independent confirmation was not possible. Detection of NMor and NPyr only occurred in systems that chloraminated.

Although the number of locations sampled in this survey was limited, the utilities selected do provide drinking water to a major portion of Alberta's population. The snapshot this survey provides suggests that most of Alberta's drinking water supplies do not contain concentrations of *N*-nitrosamines in excess of the ODWQS of 9 ng/L, which is encouraging from a provincial perspective. However, this survey was limited in scope because it represents only a one-time sampling, and therefore temporal/seasonal trends could not be evaluated. The carcinogenic nature of *N*-nitrosamines in association with the drinking water route of exposure warrants future survey work in Alberta as well as in other jurisdictions in Canada, where currently no NDMA data exist. Locations identified as having high *N*-nitrosamine concentrations ought to allocate resources to evaluate appropriate treatment processes and source water quality, ultimately allowing informed decisions to be made in terms of actions necessary to reduce *N*-nitrosamines in drinking water.

3.3.5 Routine water quality

Distribution drinking water samples represented a range of finished water quality parameters (**Table 3.3**). Initially, an evaluation of routine water quality parameters (pH, DOC, SUVA, NH_3-N, NO_3^-, TKN, and Organic N) was conducted to assess whether there were any trends between the water quality variables (independent) and NDMA concentrations (dependent variable). Using SPSS® version 13.0 for Windows®, scatter plots were generated between NDMA and each independent water quality variable. For analytical values reported as not detectable, a value of half the detection limit was arbitrarily chosen for graphing purposes. Results from all scatter plots suggested there were no apparent trends (data not shown). Therefore, correlation or regression analyses of the data were not warranted.

Due to the high number of non-detectable samples, particularly for NDMA, two additional statistical analyses were conducted. First, for water quality data with both

Table 3.2. Quantification of *N*-nitrosomorpholine (NMor) and *N*-nitrosopyrrolidine (NPyr) detected in select chloraminated Alberta drinking-water distribution system samples (July–September 2004).

Location	U of A[a]		IL2[b]
	Concn (ng/L) ± RSD[c] (%)		Concn (ng/L)
	NMor	NPyr	NMor
A1	< 2	4 ± 23	NA
A2	< 2	3 ± 25	2.4; 2.3
A3	< 2	< 3	NA
I	3 ± 22	< 3	2.0; 2.3

[a] NMor and NPyr determined by solid-phase extraction and GC/MS ammonia positive chemical ionization with internal standard quantification; average concentration reported, n = 3.

[b] Independent laboratory two (IL2). NMor determined by solid-phase extraction and GC/HRMS electron ionization with isotope dilution quantification (OMOE, 2004*c*); duplicate sample concentrations reported, NPyr not analyzed by IL2.

[c] Relative standard deviation.

Note: NA, Not analyzed.

detectable and non-detectable results (NH_3-N, NO_3^-, TKN, and Organic N), the variables were dichotomized and compared with NDMA using a Fisher's Exact Test (2-sided). Additionally, disinfection type (chlorination-only or chloramination) was compared with NDMA using a Fisher's Exact Test (2-sided). An *a priori* level of significance was set at $p < 0.05$, which resulted in no statistically significant associations between any of the dichotomized water quality parameters and NDMA (**Table 3.4**). Additionally, there was no difference ($p = 0.325$) between disinfection type and the detection of NDMA. A nonparametric Mann-Whitney U test was the second statistical analysis used to compare water quality data, which have all known values (pH, DOC, SUVA) and NDMA (detectable or non-detectable) (**Table 3.4**). No statistically significant associations were identified ($p < 0.05$), however, there was a trend between higher DOC ($p = 0.09$) and SUVA ($p = 0.07$) values and detectable NDMA. Though suggestive, as with any small dataset, interpretation of results must be made cautiously. Certainly, more rigorous statistical evaluations of water quality parameters and NDMA formation ought to be conducted once a more comprehensive NDMA occurrence database is developed.

3.3.6 Alternative DBPs and NDMA occurrence

Alternative modes of disinfection, such as with chloramines, are often used in place of chlorination because of the tendency to produce less chlorinated DBPs such as THMs and HAAs (Diehl et al., 2000). In Canada only THMs are currently regulated (FPT-CDW, 2004), while in the United States both THMs and HAAs are regulated (USEPA, 1998). Ironically, in an attempt to reduce regulated DBP concentrations, alternative disinfection processes such as chloramination have in some cases been found to generate unregulated DBPs such as *N*-nitrosamines that may be of equal or greater health concern compared to THMs and HAAs. Additionally, iodoacid DBPs have recently been identified in chloraminated drinking waters, which were derived from high bromide and iodide containing source waters (Plewa et al., 2004). The iodoacid DBPs tested exhibited increased cytotoxicity and genotoxicity compared to bromoacetic and chloroacetic acids, which are regulated HAAs in the United States.

Accurate exposure data are needed to better estimate health risks posed by alternative DBPs. Currently, limited public data are available to assess the magnitude and frequency of NDMA occurrences in drinking water. Most recently, Barrett et al.

Table 3.3. Water quality parameters of select Alberta drinking-water distribution system samples (July–September 2004).

Location	pH[a]	Total Cl[b] (mg/L)	DOC[c] (mg/L)	UV_{254}[d] (cm^{-1})	SUVA[e] (L/mg m)	NO_3^{-}[f] (mg/L)	NH_3-N[g] (mg/L)	TKN[h] (mg/L)	Org N[i] (mg/L)
A1	8.1	1.1	8	0.17	2.1	0.9	0.70	1.8	1.1
A2	8.3	0.4	8	0.15	1.8	0.8	0.74	1.6	0.86
A3	8.0	1.4	8	0.15	1.9	0.5	0.88	1.9	1.00
B	8.2	1.6	5	0.061	1.2	0.2	0.77	1.0	0.23
C	7.3	NA	3	0.077	2.6	0.1	0.43	0.5	0.07
D	7.2	1.8	6	0.10	1.7	0.2	0.31	1.0	0.69
E	7.8	0.6	6	0.21	3.5	0.2	0.42	0.8	0.38
F	7.8	1.5	3	0.035	1.2	0.2	0.33	0.4	0.07
G	8.1	1.8	5	0.074	1.5	0.1	0.34	0.8	0.46
H	7.9	1.3	2	0.032	1.6	0.2	0.54	0.6	0.06
I1	8.2	1.1	2	0.058	2.9	0.1	0.45	0.5	0.05
I2	7.8	1.5	3	0.034	1.1	0.2	0.27	0.3	0.03
J	8.0	1.1	5	0.075	1.5	0.4	0.29	1.1	0.81
K	8.1	0.6	4	0.041	1.0	0.7	<0.05	0.5	0.5
L	8.0	0.8	8	0.18	2.3	0.1	2.90	2.8	<0.2
M	8.0	0.3	7	0.22	3.1	0.1	<0.05	0.5	0.5
N	8.1	1.0	3	0.013	0.43	<0.1	<0.05	<0.2	<0.2
O	7.9	NA	4	0.028	0.70	<0.1	<0.05	<0.2	<0.2

Table 3.3. ***continued.*** **Water quality parameters of select Alberta drinking-water distribution system samples (July–September 2004).**

Location	pH[a]	Total Cl[b] (mg/L)	DOC[c] (mg/L)	UV_{254}[d] (cm^{-1})	SUVA[e] (L/mg m)	NO_3^-[f] (mg/L)	NH_3-N[g] (mg/L)	TKN[h] (mg/L)	Org N[i] (mg/L)
P	7.7	0.2	3	0.027	0.90	<0.1	<0.05	<0.2	<0.2
Q	7.6	NA	7	0.064	0.91	<0.1	<0.05	<0.2	<0.2
R	8.0	1.9	11	0.13	1.2	<0.1	<0.05	0.8	0.8
S	8.1	0.5	5	0.10	2.0	0.1	<0.05	0.4	0.4
T	7.5	1.9	4	0.079	2.0	<0.1	<0.05	0.3	0.3

[a] APHA 4500-H^+, pH value.

[b] APHA 4500-Cl D, Forward Amperometric Titration Method (combined chlorine residual).

[c] DOC, Dissolved Organic Carbon; APHA 5310 C, Persulfate-Ultraviolet (UV) Method.

[d] UV Spectroscopy measured at 254 nm.

[e] SUVA, Specific Ultraviolet Absorption (*calculated*).

[f] APHA 4500-NO_3^- H, Automated Hydrazine Reduction Method/Colorimetry.

[g] APHA 4500-NH_3 F, Phenate Method/Colorimetry.

[h] TKN, Total Kjeldahl Nitrogen; APHA 4500-N C, Persulfate Method.

[i] Org-N, Organic Nitrogen; *calculated* as the difference between TKN and NH_3-N. If NH_3-N is < 0.05 mg/L then Org-N = TKN.

Note: All APHA methods based on (APHA, 1998).

NA, Not analyzed.

(2003) conducted quarterly monitoring of NDMA for 21 North American water treatment utilities. The maximum detected NDMA concentrations for plant effluent (n = 81) and distribution samples (n = 95) samples were 30 ng/L and 24 ng/L respectively. The median NDMA distribution system concentration for chloramination was < 2 ng/L and < 1 ng/L for chlorination.

The California Department of Health Services (CDHS) has a publicly available NDMA occurrence dataset that was developed in 1999. Several California water treatment plants in cooperation with the CDHS conducted NDMA testing of drinking water supplies (CDHS, 2002). The CDHS occurrence data, summarized in **Table 3.5**, show maximum NDMA concentrations for chloraminating plant effluents (n = 31) and distribution samples (n = 34) samples of 18 ng/L and 16 ng/L respectively.

The most extensive source of NDMA occurrence data is from Ontario, maintained by the OMOE through the auspices of the DWSP. Data collected between 1994 and 2002 for NDMA are summarized in **Table 3.6**. In addition to NDMA, the Ontario DWSP Summary Report for 2000–2002 (OMOE, 2004*a*) contains occurrence data for DENA, NMor, and DBNA, although there are no ODWQS for these species. Today, over 140 Ontario municipal water systems voluntarily participate in the DWSP. Between 2000 and 2002, 1,021 samples were analyzed for NDMA, resulting in 5 municipal drinking water systems having 23 tests exceeding 9 ng/L. Of the 23 NDMA exceedences, 15 were from one chloraminating facility (ranging from 9.3 ng/L to 19 ng/L) and the three highest overall NDMA concentrations came from a single chlorination-only facility (maximum 66 ng/L). After extensive testing, the utility with 15 exceedences changed their summer polymer, a blend of alum and cationic polymer containing quaternary amines, which resulted in an almost immediate decrease of NDMA concentrations below the ODWQS[1]. Some amine-based polymers such as diallyldimethylammonium chloride (DADMAC) are known to form NDMA upon chloramination (Wilczak et al., 2003) or chlorination (Kohut and Andrews, 2003).

Presently in Canada, with the exception of Ontario, there are no data publicly available that report *N*-nitrosamine occurrences in drinking water. The public reporting of water quality data is an important component for making drinking-water quality risk

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[1] Personal communication, Patrick Halevy, Water Quality Manager, City of Brantford (May 3, 2005).

Table 3.4. Statistical comparisons between routine water quality parameters and NDMA (detectable and non-detectable[a]).

Finished Water Quality Parameter	Mean ± SD[b] (Median) or % Detectable		p-value[c, d]
	NDMA Detectable (n=6)	NDMA Nondetectable[a] (n=17)	
pH	8.0 ± 0.4 (8.1)	7.9 ± 0.3 (7.9)	0.20[c]
DOC[e] (mg/L)	6.5 ± 2.1 (7.5)	4.8 ± 2.3 (4.0)	0.09[c]
SUVA[f] (L/mg m)	2.1 ± 0.66 (2.0)	1.6 ± 0.80 (1.5)	0.07[c]
NO_3^-	100 %	65 %	0.14[d]
NH_3-N	83 %	53 %	0.34[d]
TKN[g]	100 %	76 %	0.54[d]
Org-N[h]	100 %	71 %	0.27[d]

[a] Method detection limit for NDMA = 5 ng/L.

[b] Standard deviation

[c] p-value based on nonparametric Mann-Whitney U test.

[d] p-value based on Fisher's Exact test.

[e] DOC, Dissolved Organic Carbon.

[f] SUVA, Specific Ultraviolet Absorption (*calculated*).

[g] TKN, Total Kjeldahl Nitrogen.

[h] Org-N, Organic Nitrogen.

management decisions. Utilities and regulators considering switching to chloramination or other alternative forms of disinfection ought to consider source water characteristics, treatment plant designs, as well as risk trade-offs between the production of regulated but less toxic DBPs and the generation of emerging DBP classes that are potentially more toxic.

For at least one Alberta location, authentic drinking water concentrations of NDMA have been documented to persist near 100 ng/L over a period of years. In Ontario as with our present Alberta survey, some facilities appear to face serious challenges for reducing NDMA, while a majority of systems appear to have little issue with NDMA or other *N*-nitrosamines. The detection of additional *N*-nitrosamine species including DBNA in Ontario (OMOE, 2004*a*), which is a known bladder carcinogen, reinforces the importance of monitoring for several *N*-nitrosamine species. Until more is known about the key variables involved in *N*-nitrosamine formation, as well as the importance of drinking water *N*-nitrosamine exposures relative to all sources of exposure, questions will persist regarding the best way to manage *N*-nitrosamine DBP risks.

Ultimately, the priority for providing safe drinking water must be focused on remaining vigilant against pathogenic microorganisms and avoiding complacency in routine tasks, particularly when water treatment plant conditions become challenged (Hrudey and Hrudey, 2004). Water quality chemists will continue to develop faster, more simple, and more sensitive analytical technologies that will result in a continued lowering of drinking-water analyte detection limits (Ells et al., 1999). The cycle of analytical improvements followed by the identification of novel compounds in drinking water has already occurred several times and will likely continue. However, it is in seeking a balance between immediate and certain microbial pathogen risks, and the delayed and uncertain hazards that occur from exposures to DBPs, where water quality specialists and regulators must direct their efforts. The unwarranted preoccupation with readily detectable halogenated DBPs that pose little to no significant health concern must be refocused on chemical species that are toxicologically relevant and biologically plausible to cause the observed adverse human health outcomes seen in epidemiology studies, such as urinary bladder cancer.

Table 3.5. ***N*-Nitrosodimethylamine (NDMA) data from 32 California surface water treatment plants (1999) using either chloramines (20), chlorine (7), or ozone/chlorine (5) disinfection. Data reported represent 153 NDMA analyses from raw water, treatment plant and distribution sampling.**

Treatment	Sample Location	Samples (n)	Median (ng/L)	Min (ng/L)	Max (ng/L)	Samples < MDL (n)
Chlorine	Influent	11	<1	<1	9.4	8
Chlorine	Effluent	11	<1	<1	3.3	8
Chlorine	Distribution	12	<1	<1	2.5	8
Chloramine	Influent	27	<1	<1	3.9	18
Chloramine	Effluent	31	1.8	<1	18	10
Chloramine	Distribution	34	1.8	<1	16	7
Ozone and Chlorine	Influent	7	<1	<1	1.3	5
Ozone and Chlorine	Effluent	10	<1	<1	3.9	5
Ozone and Chlorine	Distribution	10	<1	<1	6.8	7

Note: Original data accessible through the California Department of Health Services website (CDHS, 2002).

Table 3.6. ***N*-Nitrosodimethylamine (NDMA) data from 179 Ontario water treatment plants (1994–2002) using either chloramines (21), chlorine (157), or ozone/chlorine (1) disinfection. Data reported represent 3063 NDMA analyses from raw water, treatment plant and distribution sampling collected through Ontario's Drinking Water Surveillance Program.**

Treatment	Sample Location	Samples (n)	Median (ng/L)	Min (ng/L)	Max (ng/L)	Samples < MDL (n)
Chlorine	Influent	851	<1	<1	8	606
Chlorine	Effluent	1429	<1	<1	40	835
Chlorine	Distribution	282	<1	<1	66	182
Chloramine	Influent	142	<1	<1	6.7	89
Chloramine	Effluent	277	1.3	<1	65	111
Chloramine	Distribution	76	2.2	<1	18	16
Ozone and Chlorine	Influent	2	<1	<1	<1	2
Ozone and Chlorine	Effluent	2	<1	<1	<1	2
Ozone and Chlorine	Distribution	2	<1	<1	<1	2

Note: Data were requested and obtained through the Water Monitoring Section, Environmental Monitoring and Reporting Branch, Ontario Ministry of the Environment.

3.4 Conclusions

In our *N*-nitrosamine survey an SPE manifold system was successfully implemented, reducing sample-processing times. An increased number of quality control samples were included, in addition to triplicate analysis of most authentic drinking-water samples. Overall, NDMA was detected in a limited number of distribution systems (30%) and found at an exceptionally elevated concentration (up to 11 times the ODWQS) at one location. Most NDMA concentrations were associated with facilities that chloraminated. However, one location using chlorination-only was also found to have an NDMA concentration above the ODWQS. Overall, Alberta NDMA distribution concentrations rarely exceeded the ODWQS. Moreover, two other *N*-nitrosamines (NPyr and NMor) were identified at multiple locations. Although this work targeted water treatment plants serving the majority of Alberta's population, it was still limited because it represents only a point estimate and was not capable of determining temporal or seasonal trends. Continued monitoring of *N*-nitrosamine concentrations in drinking water will help address uncertainties that exist regarding potential fluctuations over time due to variations in source water quality and water treatment operations.

Canadian efforts are required to determine a national *N*-nitrosamine occurrence dataset. A prudent starting point for evaluating Canadian NDMA exposures in drinking water would be to assess past experiences from Ontario as well as to make use of the extensive DWSP database. Additional DBP research also needs to focus on the identification and control of precursors that can lead to extreme *N*-nitrosamine concentrations in drinking water, such as from Location 'A' in this study. From a risk management perspective, identifying utilities with elevated DBPs of health concern should provide for improved resource allocation (financial as well as intellectual) to utilities in need of controlling extreme DBP concentrations of human health significance. Understanding the occurrence of NDMA as a DBP as well as acknowledging alternative exposure pathways from sources such as diet, commercial products, and endogenous formation are critical for evaluating water quality needs and ultimately protecting public health.

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Chapter Four

Implications of Breakpoint Chlorination and Free-Chlorine Contact Time for Drinking Water *N*-Nitrosodimethylamine Concentrations[1]

4.1 Introduction

Disinfection of drinking water is one of the greatest advances in public health protection. During drinking water treatment, unintended chemical disinfection by-products (DBPs) are produced by complex reactions between oxidants (*e.g.* chlorine, chloramine, ozone, or chlorine dioxide) used for disinfection of pathogenic microorganisms and diverse groups of precursors such as: humic materials (Reckhow et al., 1990; Singer, 1999), bromide (Haag and Hoigne, 1983), iodide (Plewa et al., 2004a), and some amine-based coagulant aids (Wilzac et al., 2003; Kohut and Andrews, 2003). Although drinking water is a complex mixture of chemical constituents, with over 500 individual DBP species identified to date (Richardson, 1998), DBP research and regulatory agendas have primarily focused on chlorinated and brominated analogs of the two most abundant DBP classes: trihalomethanes (THMs) and haloacetic acids (HAAs).

Public concerns regarding adverse health outcomes resulting from increased exposure to drinking water DBPs stem from several epidemiology studies that demonstrated elevated risks of developing urinary bladder cancer (Mills et al., 1998; Villanueva et al., 2004) or adverse reproductive outcomes (Nieuwenhuijsen et al., 2000). However, in spite of significant DBP research efforts, identification of (a) plausible DBP agent(s) and mechanism(s) of action leading to bladder cancer are yet to be elucidated (Bull, 2001). Low epidemiology cancer risk estimates from lifetime exposures to DBPs are still relevant because they could translate into a significant number of cases at the population level because exposure is widespread. Given considerable uncertainty in understanding adverse health effects attributed to DBPs, drinking water research requires refocusing toward DBPs that are biologically capable of producing the observed adverse outcomes measured in epidemiology and toxicology studies.

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N-Nitrosodimethylamine (NDMA) is a non-halogenated DBP occurring in drinking water and treated wastewater (Mitch et al., 2003). *N*-Nitrosopyrrolidine and *N*-nitrosomorpholine have also been identified in drinking water (Charrois et al., 2004). As a chemical group, *N*-nitroso compounds have caused cancer in every vital tissue tested (Shank and Magee, 1981) and NDMA is a "probable" human carcinogen (USEPA, 1987). Additionally, the bladder is the site of action for several *N*-nitroso compounds in humans and rodent models (IARC, 1978; Shank and Magee, 1981). Thus, *N*-nitrosamines provide a more biologically plausible basis from which to investigate correlations between cancer endpoints and DBP exposures, compared to THMs and HAAs.

A trend amongst North American drinking water utilities is the incorporation of alternative disinfectants, such as chloramines, in order to comply with current and upcoming DBP regulations (*e.g.* Stage 2 Disinfectants and Disinfection Byproducts Rule). Though alternative disinfectants generally produce lower concentrations of THMs and HAAs (Kirmeyer et al., 2004), switching to chloramination still requires informed decision-making that considers risk trade-offs. Growing evidence suggests NDMA occurs more frequently and at higher concentrations in drinking water systems that chloraminate compared to chlorination-only systems (Najm et al., 2001; Wilczak et al., 2003; Charrois et al., 2005). Additionally, chloramination can produce other unregulated DBP classes. Some such as the halonitromethanes or certain iodoacid species have been shown more genotoxic or cytotoxic compared to regulated DBPs (Plewa et al., 2004a; 2004b). Moreover, switching from chlorine-only to chloramination can result in the release of lead into drinking water from distribution system pipes, solder and brass fittings (Edwards and Dudi, 2004) creating additional public health challenges for utilities. Chloramination risk tradeoff considerations are emerging as a critical research gap that warrants increased scrutiny and must be addressed prior to utilities adopting changes to full-scale disinfection practices.

N-Nitrosamine monitoring efforts in drinking water continue to increase and with the inclusion of NDMA and five other *N*-nitrosamines in the Unregulated Contaminant Monitoring Regulation 2 (UCMR 2) (USEPA, 2005), it is reasonable to anticipate that additional utilities will be identified as having elevated *N*-nitrosamine concentrations. With this in mind, a series of bench-top experiments was designed using raw source waters as well as partially-treated waters collected prior to disinfection, but after full-scale coagulation, flocculation, sedimentation, and filtration from two treatment plants in Alberta, Canada. Chloramination/breakpoint experiments were conducted, followed by extraction and analysis for NDMA. The main objectives of this study were to: 1) compare full-scale chloramination processes and NDMA formation, 2) explore the influence of Cl_2:NH_3-N ratios on the production of NDMA at the bench-scale and 3) identify potential treatment process options for drinking water utilities experiencing elevated NDMA concentrations, specifically varying free-chlorine contact time prior to ammonia application.

4.2 Materials and Methods

4.2.1 Reagents and standards

Methanol (AnalaR®) and dichloromethane (DCM) (Omni-Solv®) were acquired from VWR Canlab (Mississauga, ON). Hexane and reagent water (Optima Grade) as well as sodium bicarbonate and L-ascorbic acid (ACS reagent grade) were obtained from Fisher Scientific (Nepean, ON). Additionally, sodium hypochlorite (purified grade; 4%–6%) and ammonium hydroxide (ACS *Plus;* 14.8 M) were obtained through Fisher Scientific. Solid-phase extraction (SPE) materials, Ambersorb® 572 (Rohm & Haas; Philadelphia, PA) and LiChrolut® EN (Merck; Darmstadt, Germany) were supplied through Supelco (Oakville, ON) and VWR Canlab respectively. A standard solution containing nine *N*-nitrosamines, including *N*-nitrosodimethylamine (NDMA), was purchased from Supelco. Isotopically labeled standards, (98%) ([6-^{2}H] *N*-nitrosodimethylamine, NDMA-*d6* and [14-^{2}H] *N*-nitrosodi-*N*-propylamine, DPNA-*d14*) were from Cambridge Isotope Laboratories (Andover, MA).

4.2.2 Alberta water treatment plants and sample collection

4.2.2.1 City A

City A employs conventional treatment consisting of: powdered activated carbon, aeration, alum with cationic polymer (diallyldimethylammonium chloride; poly-DADMAC), clarification, lime softening, CO_2 (pH control), filtration (anthracite/sand/gravel), disinfection, and fluoride. Disinfection occurs after filtration in the following order: chlorine (gas), medium pressure UV (Sentinel® UV Disinfection System; Calgon Carbon Corporation, Pittsburgh, PA), followed immediately by aqua ammonia. The amount of free-chlorine contact time before ammonia addition is nominal (< 1 minute). Prior to February 2004 chlorine and ammonia were added simultaneously.

4.2.2.2 City B

City B employs conventional treatment consisting of: potassium permanganate, alum with cationic polymer (poly-DADMAC) and anionic polymer, clarification, filtration (granular activated carbon/sand), disinfection, and fluoride. Chlorine (gas) is added immediately after filtration but aqua ammonia is only added prior to water entering the distribution system. The time between chlorine and ammonia additions is approximately 2 to 4 hours depending on seasonal flow rates, providing substantial free-chlorine contact time. City B's ammonia addition is based on a measured free-chlorine residual, post-chlorine contact (2-4 hours), which is dissimilar from City A where the ammonia dose is based on the initial applied chlorine dose (pre-chlorine contact).

4.2.2.3 Experimental waters: raw, partially-treated and finished

Throughout our experiments we used: i) raw source water, ii) partially-treated water, and iii) finished water samples. Partially-treated water refers to samples collected at the treatment plant in City A and City B, prior to disinfection, but after full-scale coagulation, flocculation, sedimentation, and filtration. Partially-treated water allowed us to have a common disinfection treatment (chlorine and ammonia additions) while still including differences between plant processes such as cationic polymer additions and other process chemicals. Finished water refers to water collected after treatment plant disinfection, either in the clear well or within the distribution system.

All samples were collected in pre-cleaned amber glass bottles with PTFE lids. Prior to use, sample bottles were washed, rinsed with reagent water, followed by DCM and baked overnight at 170 °C. During sample collection, bottles were filled to zero headspace and 20 mg/L of powdered L-ascorbic acid (finished drinking water only) was added. Samples were immediately placed into coolers with icepacks and transported to the laboratory, where they were refrigerated at 4 °C until use, typically within 1 week.

Prior to use, raw water samples were pumped through Tygon® R-1000 tubing (Saint-Gobain Performance Plastics; Akron, OH) using a Masterflex® peristaltic pump (Cole Parmer Instrument, Vernon Hills, IL) and filtered through pre-combusted (400 °C for 4 hrs) Whatman® (UK) glass microfibre filters (GF/F; particle retention > 0.7 μm; 142 mm). Filters were placed in an in-line stainless steel holder (142 mm; Geotech Environmental Equipment; Denver, CO). Filtrates were then used in a series of breakpoint chlorination experiments. If left unfiltered raw water samples would clog the solid-phase extraction (SPE) materials during *N*-nitrosamine extraction, increasing processing times to an unacceptable level as well as preventing complete drying of SPE phases, which was critical for analyte extraction.

4.2.3 Disinfection experiments

4.2.3.1 Preparation of chlorine and chloramine solutions

All disinfection experiments were conducted in pre-cleaned 1L amber glass bottles at room temperature (22 ± 1 °C). Glassware, for disinfection experiments, was pretreated (> 12 hours) with sodium hypochlorite (50 mg/L) to remove chlorine demand, rinsed with copious reagent water and oven baked overnight (170 °C).

Stock-standard chlorine solutions were prepared by volumetrically diluting purified grade sodium hypochlorite into demand-free reagent water. Stock-standard ammonia solutions were prepared daily by volumetrically diluting ammonium hydroxide into reagent water. Stock-standard solutions were aged for 30 minutes in the dark before use.

4.2.3.2 Breakpoint chlorination experiments

At the bench-scale, stock-standard ammonia (1 mg/L NH_3 (0.059 mM)) was first added into rapidly stirring City A and City B raw and partially-treated waters. Stock-standard hypochlorite solutions were then diluted into the pre-ammoniated waters at Cl_2 doses ranging from 1mg/L–20 mg/L (0.014 mM–0.28 mM). After 30 minutes of mixing, resultant solutions were analyzed for free and combined chlorine residual concentrations using amperometric forward titration procedures based on *Standard Methods* 4500-Cl D (APHA, 1998). Automated titrations were made using an AutoCAT™ 9000 (Hach Company; Loveland, CO). Chlorine reference solutions, obtained from the Hach Company as well as stock-standard solutions were diluted in reagent water and analyzed to ensure accurate method performance. After completing chlorine residual measurements each reaction vessel was stored in the dark for an additional 2 hours, prior to quenching with L-ascorbic acid. Once quenched, bottles were stored at 4 °C until extraction and analysis of NDMA, which was typically 1 to 3 days.

4.2.4 *N*-Nitrosamine analysis

Samples (500 mL) were extracted for NDMA by our previously described modified method (Charrois et al., 2005). Briefly, glass SPE cartridges were packed with: 350 mg LiChrolut® EN (bottom), 500 mg Ambersorb® 572 (middle), and a wad of glass wool (top). The SPE materials were washed and conditioned (Charrois et al., 2004). Samples were continuously transferred through individual PTFE transfer lines from sample bottles, through SPE materials, and into the glass collection tank. Flow rates were 3–6 mL/min under slight vacuum. The SPE materials were dried under vacuum followed by elution with DCM (15 mL). Eluates were collected in 50 mL glass extraction tubes and concentrated to final volume of ca. 100 µL using a TurboVap®II Concentration Workstation (Zymark Corporation (now Caliper Life Sciences); Hopkinton, MA).

Sample extracts were chromatographically separated and quantitatively analyzed for NDMA using an internal standard procedure with GC/MS ammonia positive chemical ionization (PCI), based on our previous method (Charrois et al., 2004). Area responses were used to calculate relative response factors (RRFs) for NDMA using the internal standard DPNA-*d14*. Extraction efficiency was determined by measuring the percent recovery of the surrogate standard, NDMA-*d6*, which was added to each eluent just prior to extraction. All NDMA data reported were recovery corrected.

4.3 Results and Discussion

4.3.1 Quality assurance / quality control (QA/QC)

It was difficult to obtain NDMA-free reagent water, so during NDMA analysis, a total of seven laboratory reagent-water blanks were analyzed. The calculated mean concentration of NDMA was 1.1 ± 0.7 ng/L. Six laboratory fortified blanks (LFB; 10 ng/L) were used to evaluate the analytical method performance, with a mean accuracy for NDMA of 103 ± 8%. The calculated method detection limit (MDL) incorporated measured responses from the blank samples, which resulted in a more conservative MDL compared to an estimate based only on a standard deviation from LFBs. Our calculated MDL was 3 ng/L [MDL = Average Blank Concentration + $t_{(N-1,\ 1-\alpha=0.99)} \times S_{Blk}$; where t is the one-tail Student t-test value for N-1 degrees of freedom, and S_{Blk} is the standard deviation of independent NDMA reagent-water blank concentrations (n = 7)].

4.3.2 NDMA distribution system trends

Periodic sampling for NDMA was conducted at locations throughout City A's distribution system (A1–A4) as well as one location in City B between July 2003 and June 2005. Location A1 was at the water treatment plant and represents finished water. Location A2 was a mid-distribution location. Locations A3 and A4 were at remote ends of the distribution system, representing the highest residence times. Location A4 was a continuous part of City A's distribution system but the sampling location was under the jurisdiction of a separate village, 15 km outside City A. A unique feature of Location A4 was, prior to drinking water entering the village's distribution system free chlorine was added to boost the disinfectant residual from City A. The sampling location in City B was a mid-distribution location.

Figure 4.1 illustrates daily applied-polymer doses and NDMA concentrations for City A. Throughout this paper applied polymer dose refers to the delivered treatment plant dose of the cationic polymer, diallyldimethylammonium chloride (poly-DADMAC), 20% of which was active ingredient (w/w). Reactions of poly-DADMAC with chlorine (Kohut and Andrews, 2003) and chloramines (Wilczak et al., 2003) can yield NDMA.

Applied poly-DADMAC doses for City A ranged from 3–14 mg/L (median = 5 mg/L). The highest NDMA concentration measured was 180 ng/L at location A2 (July 2003), which was preceded 12-days earlier by a 4-week duration of City A's highest reported polymer dose (14 mg/L). Overall, drinking water NDMA concentrations were relatively high throughout the study, however NDMA did appear to respond at least in part to changes in poly-DADMAC doses (**Figure 4.1**). In September 2003 sampling began for remote distribution locations (A3 and A4). Increasing NDMA concentrations were observed with increasing distribution system residence times, which was consistent with other reports (Barrett et al., 2003; Wilczak et al., 2003). Variable NDMA concentrations likely reflected changes in source water quality such as total organic carbon (TOC) concentrations as well as daily treatment plant practices.

In chloraminating facilities Wilczak et al. (2003) identified: recycled filter backwash supernatant, cationic polymer dose, and free chlorine contact time as full-scale treatment parameters related to NDMA formation. Neither City A nor City B recycled filter backwash. City A and City B use the identical poly-DADMAC formulation.

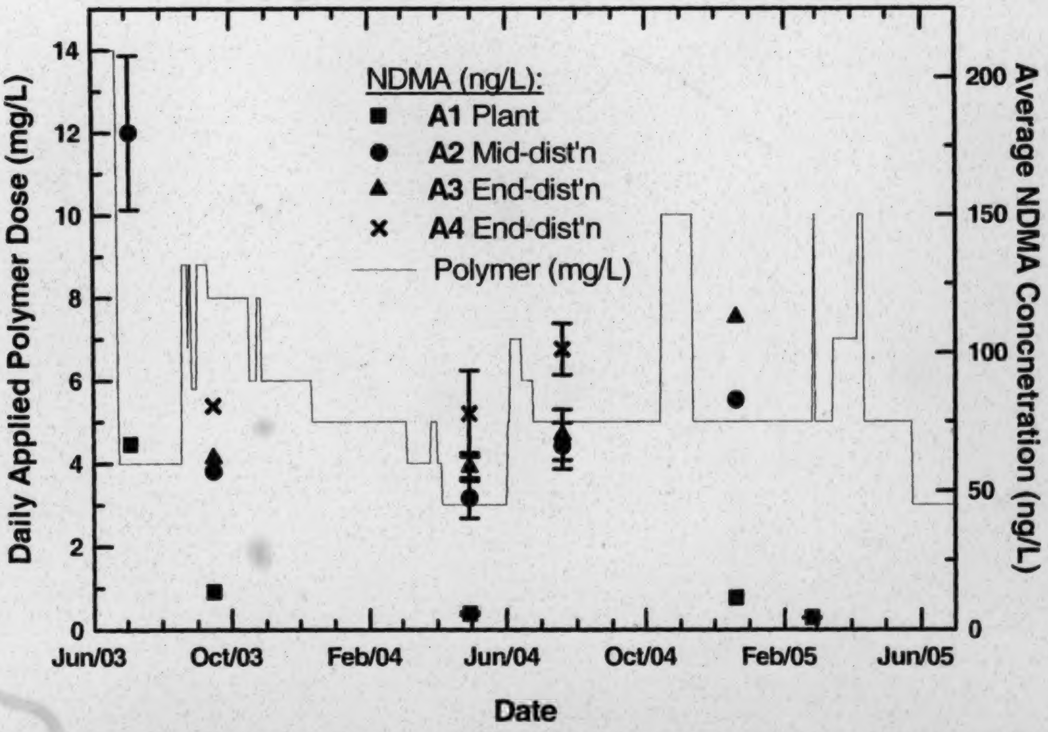

Figure 4.1. Temporal trends at City A's drinking water treatment plant for daily-applied diallyldimethylammonium chloride (poly-DADMAC) cationic polymer (mg/L) and *N*-nitrosodimethylamine (NDMA; ng/L) concentrations between July 2003 and July 2005. Applied polymer refers to actual dose of product, which was 20% active ingredient by mass. Note: when present vertical bars for NDMA indicate standard deviation from mean (n=3); all NDMA concentrations were recovery corrected using NDMA-*d6* as a surrogate standard.

However, high polymer dose alone was not a sufficient condition for elevated NDMA formation, as observed in City B. Throughout the study (July 2003–June 2005), City B's NDMA distribution concentrations were always an order of magnitude or more lower than City A's. In City B NDMA distribution system concentrations were not detected over 5 ng/L, even when the applied dose of poly-DADMAC was higher than City A's all-time maximum. City B's applied polymer dose could reach 25 mg/L. Thus additional conditions appear necessary for the formation of NDMA in chloraminated distribution systems, possibly related to practices at the treatment plant level.

Routine water treatment processes such as flocculation are inefficient at removing polar nitrogen compounds such as aliphatic and alicyclic amines from surface water sources (Pietsch et al., 2001). The aliphatic amine, dimethylamine (DMA) is a known precursor of NDMA (Choi and Valentine, 2002; Mitch and Sedlak, 2002). Pietsch et al. (2001) found ozonation was most suitable for removing polar nitrogen compounds. Several apparent differences in treatment plant practices between City A and City B may partially account for the disproportionately high NDMA values detected in City A. Firstly, City B applied potassium permanganate for controlling high manganese and iron as well as for managing taste and odour issues, while City A did not. Permanganate may pre-oxidize precursors prior to chloramination, reducing NDMA formation potential. City B's filters potentially were more efficient at removing residual poly-DADMAC and thereby reducing NDMA precursors, though this was not directly evaluated. Finally, differences in the amount of free-chlorine contact time used prior to ammoniation may explain differences in NDMA concentrations. Free-chlorine contact time in City B was 2–4 hours compared to less than 1 minute in City A. Wilczak et al. (2003) demonstrated a period of a few hours of free-chlorine contact prior to chloramination could reduce NDMA, however, concentrations were generally low (< 10 ng/L), making difficult the differentiation between true treatment differences and analytical variation at low concentrations.

4.3.3 Breakpoint chlorination experiments

Breakpoint chlorination experiments were conducted using raw and partially-treated waters from City A and City B, in order to evaluate Cl_2:NH_3-N ratios on NDMA formation. Resulting NDMA concentrations were plotted on breakpoint curves for both City A and City B raw and partially-treated waters (**Figures 4.2 and 4.3**). All NDMA

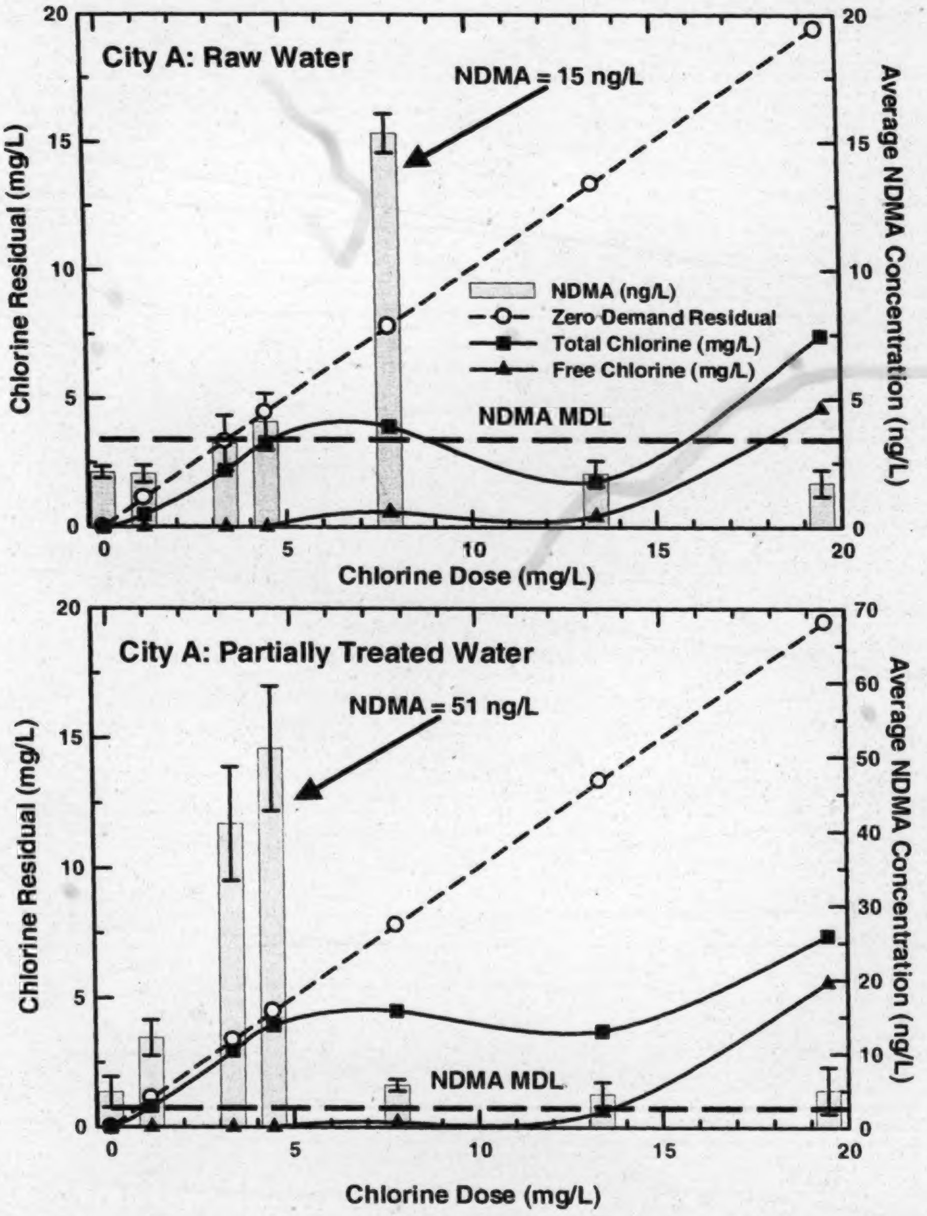

Figure 4.2. *N*-Nitrosodimethylamine (NDMA; ng/L) formation during breakpoint chlorination of City A raw water and partially-treated waters. All NDMA concentrations reported as mean ± standard deviation (n=3); all NDMA concentrations were recovery corrected using NDMA-*d6* as a surrogate standard; calculated method detection limit (MDL) = 3 ng/L. Raw water breakpoint = 2.4:1 $Cl_2:NH_3$-N; partially-treated water breakpoint 2.3:1 $Cl_2:NH_3$-N (molar ratio).

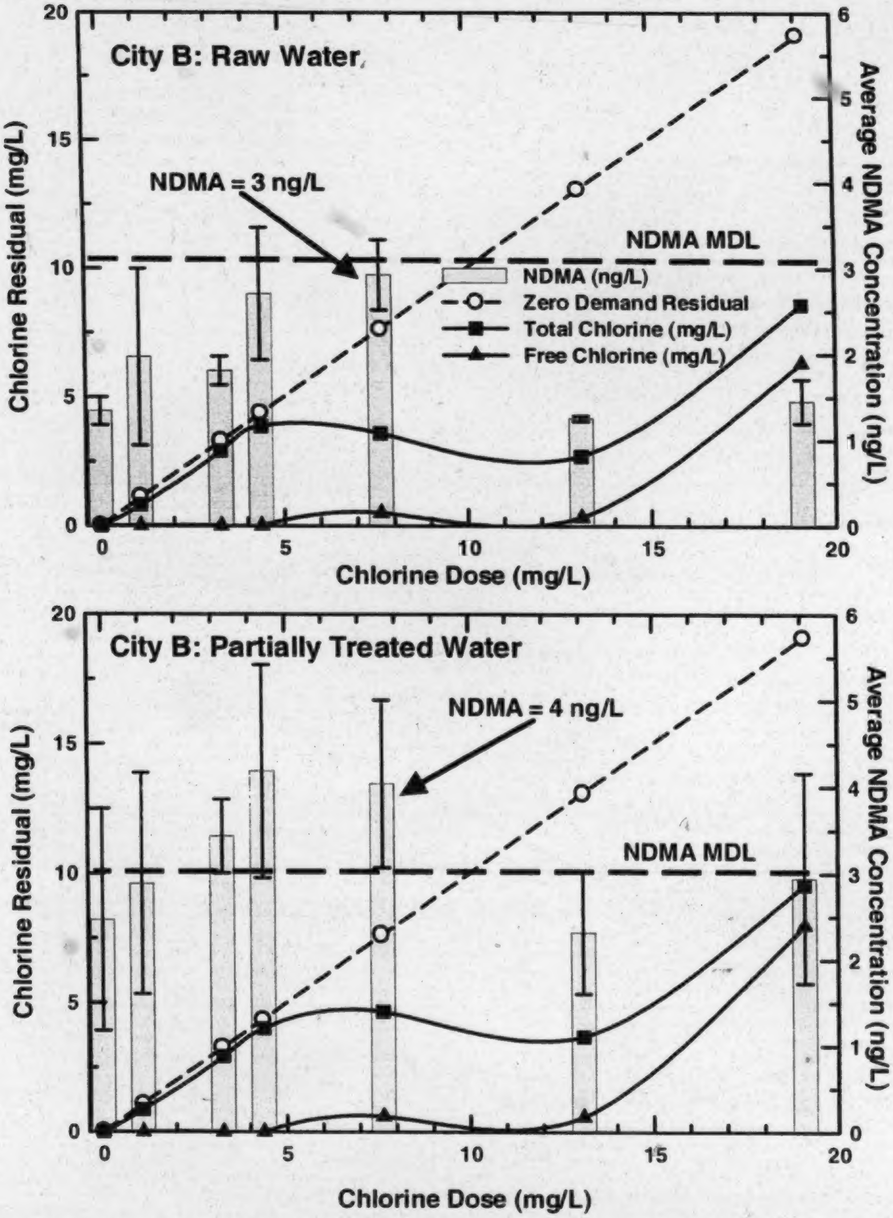

Figure 4.3. *N*-Nitrosodimethylamine (NDMA; ng/L) formation during breakpoint chlorination of City B raw water and partially-treated waters. All NDMA concentrations reported as mean ± standard deviation (n=3); all NDMA concentrations were recovery corrected using NDMA-*d6* as a surrogate standard; calculated method detection limit (MDL) = 3 ng/L. Raw water breakpoint = 1.9:1 Cl_2:NH_3-N; partially-treated water breakpoint 2.0:1 Cl_2:NH_3-N (molar ratio).

data reported were recovery corrected and expressed as the average ± standard deviation (n=3).

Bench-scale chloraminating of raw water sources allowed an investigation into NDMA precursors in natural organic matter (NOM) as well as excluding variable water treatment practices between locations. In terms of NDMA formation, source water composition differences clearly exist, however, major differences were not captured by routine monitoring parameters (**Table 4.1**). In breakpoint experiments for raw water, NDMA concentrations peaked at 15 ± 0.8 ng/L in City A while in City B NDMA was measurable but always below our calculated method detection limit of 3 ng/L. Gerecke and Sedlak (2003) demonstrated NDMA can form from NOM precursor materials, however, much higher monochloramine (1 mM) concentrations and longer reaction times (7 days) were used compared to the conditions used herein. Raw surface water sources were similar with each having high dissolved organic carbon (DOC) concentrations (City A= 19 mg/L and City B = 16 mg/L). City A raw water had a higher SUVA value (2.5 L/mg m) compared to 1.7 L/mg m for City B, suggesting a slightly higher humic component. Source waters are located 40 km apart within the same watershed and City A is downstream of City B. No obvious explanations based on source water composition were identified that could account for differences in NDMA formation between City A and City B raw water.

In City A both raw and partially-treated water NDMA concentrations peaked in the sub-breakpoint region. Concentrations of NDMA in partially-treated water peaked at 51 ± 8.3 ng/L, corresponding to a Cl_2: total NH_3-N molar ratio of 0.8:1 while the raw water molar ratio was 1.5:1. Partially-treated water from City A received a poly-DADMAC dose of 5 mg/L. City A, where NDMA concentrations were continuously elevated, maintained a constant treatment plant chloramine dosing ratio of 0.8:1 molar ratio (Cl_2: NH_3-N), throughout the entire study period. Average pH values measured during City A chloramination reactions were 7.9 ± 0.1 and 9.1 ± 0.1 for raw and partially-treated water respectively. Extrapolating from **Figure 4.2**, breakpoints for raw and partially-treated City A water occurred at Cl_2: total NH_3-N molar ratios of 2.4:1 and 2.3:1 respectively.

Table 4.1. Water quality parameters for bench-top disinfection experiments.

Sample	pH[a]	DOC[b] (mg/L)	UV_{254}[c] (cm^{-1})	SUVA[d] (L/mg m)	NO_3^-[e] (mg/L)	NH_3-N[f] (mg/L)	TKN[g] (mg/L)	Org N[h] (mg/L)	Poly-DADMAC Dose[i] (mg/L)
City A Raw	7.8	19	0.48	2.5	0.8	0.23	2.1	1.9	NA
City A PT[j]	8.8	12	0.26	2.2	0.7	0.24	1.5	1.3	5
City B Raw	7.9	16	0.27	1.7	0.3	0.38	1.8	1.4	NA
City B PT[j]	7.2	8	0.14	1.7	0.2	0.37	1.2	0.83	18

[a] APHA 4500-H^+, pH value.

[b] DOC, Dissolved Organic Carbon; APHA 5310 C, Persulfate-Ultraviolet (UV) Method.

[c] UV Spectroscopy measured at 254 nm.

[d] SUVA, Specific Ultraviolet Absorption (*calculated*).

[e] APHA 4500-NO_3^- H, Automated Hydrazine Reduction Method/Colorimetry.

[f] APHA 4500-NH_3 F, Phenate Method/Colorimetry.

[g] TKN, Total Kjeldahl Nitrogen; APHA 4500-N C, Persulfate Method.

[h] Org-N, Organic Nitrogen; *calculated* as the difference between TKN and NH_3-N.

[i] diallyldimethylammonium chloride (poly-DADMAC) dose applied at treatment plant; 20% of which was active ingredient (w/w).

[j] PT = Partly treated water.

Note: NA = Not applicable.

All APHA methods based on (APHA, 1998).

Concentrations of NDMA formed in City B partially-treated water were significantly less compared to City A. Bench-scale chloramination of City B partially-treated water, which received a higher applied cationic polymer dose (18 mg/L), resulted in a maximum NDMA concentration of 4 ± 1.2 ng/L, which corresponded to a Cl_2: total NH_3-N molar ratio of 0.6:1. Average pH values measured for City B chloramination reactions were 7.8 ± 0.04 and 7.1 ± 0.2 for raw and partially-treated water respectively. Breakpoints extrapolated from **Figure 4.3** were 1.9:1 for raw and 2.0:1 for partially-treated water (Cl_2: total NH_3-N molar ratio).

Beyond the breakpoint, the amount of NDMA formed was not significantly different from raw water blanks or partially-treated blank values in both City A and City B (average blank values = 2.7 ± 1.5 ng/L). Mitch and Sedlak (2002) demonstrated reduced NDMA formation when hypochlorite was added in excess of the breakpoint to wastewaters or to solutions of DMA and ammonia in deionized water, however, solution concentrations of disinfectants were far beyond practical dosing limits used in drinking water treatment plants. Under ideal conditions monochloramine formation is maximized at a Cl_2: NH_3-N molar ratio of 1:1 (pH =8.3) and the theoretical breakpoint occurs at a ratio of 1.5: 1 (White, 1999). Barrett et al. (1985) reported a range of breakpoint values in drinking water ranging from 1.4:1 to 3.2:1 (Cl_2:NH_3-N molar ratio). Peak NDMA concentrations in City A and City B partially-treated water occurred below the theoretical 1:1 (Cl_2: NH_3-N molar ratio) monochloramine maximum. One limitation of using total chlorine measurements is the true identities of all titrated species are unknown and likely include a complex organic chloramine mixture. In terms of water treatment practice, ammonia dose and the resulting Cl_2: NH_3-N ratio appeared to be critical parameters related to NDMA formation.

4.3.4 Free-chlorine contact experiments

Several suggestions regarding the importance of chlorine and ammonia addition sequences and the formation of NDMA have been made (Wilczak et al., 2003; Schreiber and Mitch, 2005). As a potential treatment process option for reducing NDMA in City A, the influence of an extended free-chlorine contact time at the bench-scale was explored. Breakpoint conditions that produced peak NDMA concentrations in partially-treated City A water were selected as a starting point for evaluating the effect of free-chlorine contact time on NDMA production. City B was not selected for further experiments, given low NDMA distribution system levels.

A 2 hour free-chlorine contact period was chosen for bench-scale reactions because it approximated conditions used in City B, where NDMA concentrations were relatively low (< 5 ng/L). Chloramination of City A partially-treated water at 4 mg/L (0.8:1 Cl_2: total NH_3-N molar ratio) with no free-chlorine contact time yielded 51 ± 8.3 ng/L NDMA. A variation of the 4 mg/L chloramine dose, 2 mg/L free chlorine (2 hours) followed by 2 mg/L chloramine, resulted in lower NDMA levels (16 ± 3.5 ng/L). However, when a higher pre-ammoniation dose of free chlorine (4 mg/L) was applied for 2 hours followed by a chloramine dose of 4 mg/L, 3 ± 0.7 ng/L of NDMA formed, which was a significant NDMA reduction (up to 93%), compared to no free-chlorine contact time (**Figure 4.4**). There was no significant difference between the high pre-ammoniation dose, free-chlorine only (4 mg/L) and the blanks for partially-treated water (no chlorine or ammonia added). Thus for City A's partially-treated water, a 2 hour period of free-chlorine contact before ammonia addition offers a treatment option for reducing NDMA, however, consideration of regulated DBPs was not included as part of this study.

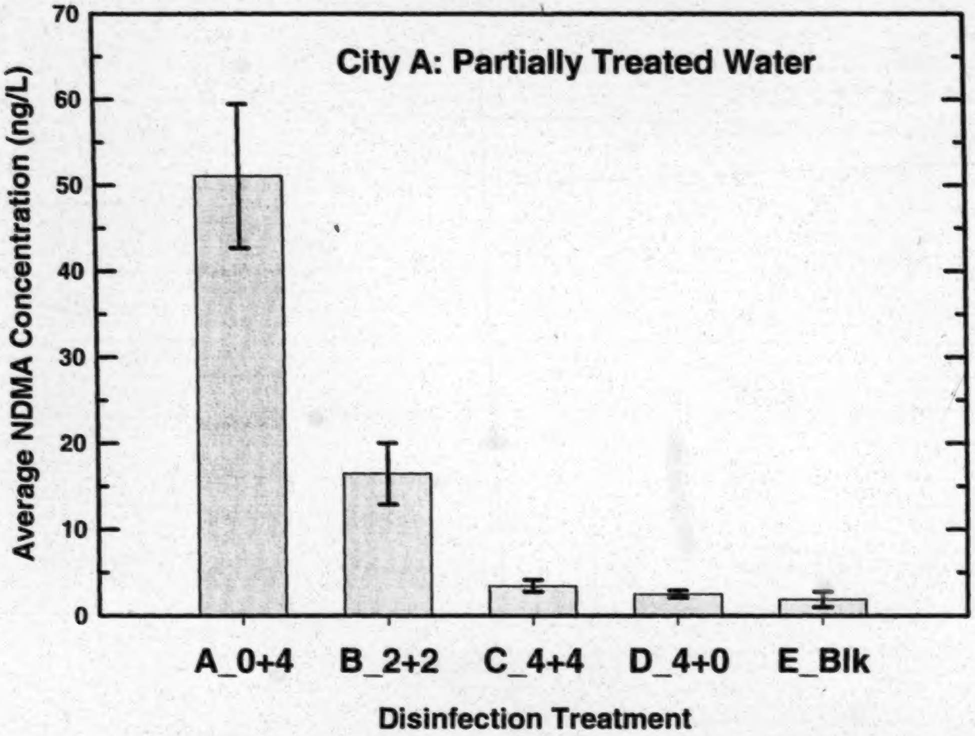

Figure 4.4. *N*-nitrosodimethylamine (NDMA; ng/L) formation during bench-scale disinfection of City A partially treated water with variable free-chlorine contact times prior to chloramination. Treatments A, B, and C were: 0, 2 and 4 mg/L free-chlorine (2 hours) followed by 4, 2, and 4 mg/L chloramines (2.5 hours) respectively. Treatment D was 4 mg/L free-chlorine only (2.5 hours) and Treatment E was City A partially-treated water only. All NDMA concentrations reported as mean ± standard deviation (n=3); all NDMA concentrations were recovery corrected using NDMA-*d6* as a surrogate standard; calculated method detection limit (MDL) = 3 ng/L.

4.4 Conclusions

Using alternative disinfectants is not entirely risk free. Chloramination may preferentially form more toxic DBPs, such as NDMA, compared to chlorine-only treatments. From our investigation of two full-scale chloraminating water treatment plants as well as from bench-scale experiments using realistic disinfection conditions we conclude:

- NDMA distribution system concentrations are variable and when detected tend to increase with increasing distribution residence time, which has implications for exposure assessments and sample collection practices.
- High cationic polymer (poly-DADMAC) doses were not a sufficient condition for NDMA formation. Treatment plant practices (*e.g.* timing of disinfectant additions) also appear to influence NDMA concentrations;
- In bench-scale experiments, increasing chlorine concentrations with a constant ammonia dose resulted in maximum NDMA formation being measured within the sub-breakpoint region of the disinfection curve. Chloramination of the partially-treated waters tested produced peak NDMA concentrations at Cl_2: NH_3-N molar ratios ranging from 0.6:1 to 0.8:1;
- Bench-scale chloramination conditions having a period of free-chlorine contact (2 hours) before chloramination clearly resulted in significant reductions of NDMA (up to 93 %), compared to no free-chlorine contact time in City A's partially-treated water.

Source water and treatment plant processes are location dependent. Utilities considering incorporating alternative disinfectants ought to conduct bench-scale testing prior to initiating full-scale changes to assess: i) DBP formation potentials including NDMA, ii) whether a period of free-chlorine contact might decrease NDMA levels if problematic, and iii) risk trade-offs between potential adverse human health effects and formation of regulated and unregulated DBP species.

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Chapter Five

N-Nitrosamines in Drinking Water: Are We Really Managing Human Health Risks?[1]

5.1 Introduction

One of the most successful public health measures ever implemented was the disinfection of drinking water by chlorination (Bull et al., 1995; Fawell et al., 1997). Disinfection of drinking water began at a time when infectious diseases were the main causes of illness and death. However, even today episodes of drinking water contamination by pathogenic microbial agents such as Walkerton and North Battleford continually reminded us that complacent attitudes towards drinking water quality can compromise the public's health and must not be tolerated. The first priority for drinking water providers must be to ensure safety from pathogenic microorganisms, such as *Escherichia coli* and *Campylobacter* as well as *Giardia* and *Cryptosporidium* species, through the effective use of disinfectants and fine particle removal.

Disinfection of drinking water, however, may not be entirely risk-free. Disinfection by-products (DBPs) result from reactions between oxidants (*e.g.* chlorine, chloramines, ozone, chlorine dioxide) and/or UV irradiation with diverse groups of precursors within source water (*e.g.* natural organic matter, bromide, as well as anthropogenic compounds). Disinfection reactions can be simplified by the following equation:

$$\textbf{Disinfectant + Precursors} \rightarrow \textbf{DBPs} \qquad \textbf{(Equation 5.1)}$$

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[1] A version of this chapter was presented as an extended abstract at the Canadian Water and Wastewater Association's 11th Canadian National Conference and 2nd Policy Forum on Drinking Water, Calgary, Alberta (April 3–6, 2004) as: Charrois, J.W.A. and Hrudey, S.E. 2004. Emergence of *N*-Nitrosamines as Disinfection By-Products: A Canadian Perspective. pp. 9.

The first class of DBPs identified was the trihalomethanes (THMs). Chloroform, the most abundant THM in drinking water, was first identified in 1974 (Bellar et al., 1974). Shortly after the identification of THMs in drinking water, a report published by the National Cancer Institute identified chloroform as a carcinogen (NCI, 1976). Exposure to chloroform in drinking water coupled with the fact chloroform was deemed a carcinogen, resulted in major public health concerns that still exist today. Moreover, a strong impetus for DBP regulatory action and research initiatives was created.

Since identifying chloroform in drinking water, the water industry has been preoccupied with halogenated DBPs. In epidemiology studies, urinary bladder cancer is the cancer associated most consistently with exposures to disinfected drinking water. Much of the adverse health-effects research has focused on exposures to two main classes of DBPs: THMs and haloacetic acids (HAAs). A Health Canada Expert Working Group concluded that it was possible to probable that chlorinated by-products pose a significant risk to the development of cancer, particularly bladder cancer (Mills et al., 1998). Yet the same consensus report found that the identified halogenated by-products could not explain the relative risks estimated from epidemiology studies. Another expert panel assembled by Health Canada in 2002 agreed that total THMs can be a surrogate for exposure to chlorinated DBPs in epidemiology studies, however, the "…total THM level is often driven by chloroform, the predominant THM, and toxicological evidence to date does not support a causal relationship between chloroform and adverse health outcomes at currently regulated drinking water exposure levels" (SENES/Global Tox, 2003; pp. S2).

Chloroform, typically the most abundant THM in drinking water, is the compound drinking water researchers and regulators continually focus on. The USEPA's Weight-of-Evidence Characterization for Human Carcinogenicity and Chloroform (2004) document states that chloroform is likely to be carcinogenic under high exposure conditions that lead to cytotoxicity and regenerative hyperplasia. Accordingly at low exposure concentrations, such as those typical in drinking water, chloroform is not believed to be carcinogenic.

Major research and regulatory efforts have focused on THMs and HAAs because of the availability of analytical instrumentation to detect these groups of compounds in water as well as their relatively high abundance in drinking water. The identification of

DBPs has closely paralleled advances in analytical chemistry. As detection technology and analytical methods improved, so did the ability to identify and quantify new compounds in drinking water. Furthermore, the rapid advancement of analytical techniques, namely gas chromatography coupled with mass spectrometry (GC/MS) lead to the development of several EPA Standard Methods, which are used for screening select groups of "target compounds". Unfortunately, an over-reliance on accepted analytical methods resulted in regulators, researchers and the water industry being preoccupied with halogenated compounds resulting from disinfection practices. Long lists of detected DBPs have been generated. Today hundreds of individual DBPs, representing several chemical classes, have been identified (Krasner et al., 1989; Richardson, 1998). Considering all the possible combinations of organic reactions that could potentially occur and the continual improvements in analytical detection limits, a list of all possible DBPs could be almost infinite. Too often health significance becomes implied by the mere detection of a compound. Detection limits are arbitrary boundaries between what can be "seen" and what may or may not be present in a sample. The desire to push detection limits lower must be balanced by the realization that not all detectable compounds are of health significance, a critical detail when considering the development of water quality guidelines. In the case of DBPs a better approach to identifying possible causal agents for the observed apparent increase in urinary bladder cancer risk is needed.

Drinking water guidelines seek to balance immediate and certain pathogenic microbial risks with delayed and uncertain hazards that may occur from exposures to DBPs. After 30 years of halogenated DBP research, water quality priorities in the drinking water industry need to be expanded to include more that than just halogenated compounds. I propose a refocusing of research priorities to look for novel DBPs that result from disinfection processes and are more toxicologically potent than THMs and HAAs.

5.2 Emergence of NDMA as a DBP

N-Nitrosamines are an emerging group of compounds worthy of further research because of their detection in drinking water and wastewater. As a chemical class, *N*-nitroso compounds have produced malignant tumors in every vital tissue tested (Shank and Magee, 1981). For example, *N*-nitrosodimethylamine (NDMA) is a carcinogen receiving a lot of recent attention as a DBP. Occurrences of NDMA in water have been linked to anthropogenic sources such as leaking liquid rocket fuel tanks as well as formation from precursors such as: dimethylamime; cationic polymers used as coagulation aids; ion-exchange resins with amine functional groups; wastewater effluents as well as herbicides, fungicides and cationic metal chelators containing dithiocarbamates.

Interest in NDMA as a DBP began in 1989 after its discovery in Ohsweken, Ontario (Jobb et al., 1994). Subsequently, around 1999, NDMA was identified in drinking waters throughout California (CDHS, 2004). There are no federal drinking water standards for NDMA in Canada or the United States, only Ontario's Drinking Water Quality Objective (ODWQS of 9 ng/L (OMOE, 2003), and California's Notification Level (NL) of 10 ng/L (CDHS, 2005). The United States Environmental Protection Agency (USEPA) established a 1×10^{-6} upper-bound lifetime cancer risk for NDMA in drinking water of 0.7 ng/L (USEPA, 1987). If exposures to *N*-nitrosamines in drinking water are shown to be a human health risk, they will need to be controlled wherever they are found to occur at unacceptably high levels.

Research on NDMA indicates it can form in drinking water and wastewater after chlorination (Mitch and Sedlak, 2002) or chloramination (Najm and Trussell, 2001; Choi and Valentine, 2002). Typical NDMA concentrations in drinking water are in the sub-ng/L to 10 ng/L range (parts-per-trillion). However, in at least one Canadian location, NDMA drinking water concentrations of 180 ng/L were recently detected (Charrois et al., 2004). Wastewater concentrations are typically in the hundreds of ng/L range (Mitch and Sedlak, 2002).

5.3 NDMA Exposures

N-Nitrosodimethylamine has been detected in tobacco, food (*e.g.* cheeses, smoked meats and canned fruit), beverages (*e.g.* beer, milk and drinking water) as well as cosmetics. In 1992 the Ontario Ministry of the Environment (OMOE) estimated the total daily intake of NDMA from food was 200 ng/day for an average Canadian. In 2002 the World Health Organization (WHO), using primarily Canadian data, projected the daily intake (air, food and water) of NDMA for people aged 20-60 as 250-1100 ng/day. Over the last few decades, many changes have occurred in the manufacturing processes of foodstuffs and commercial products. An updating of basic NDMA exposure data would help reduce uncertainties for any future risk assessments. Additionally, any evaluation of DBP risks from drinking water NDMA exposures must be considered in relation to all sources of *N*-nitrosamine.

5.4 NDMA Cancer Risk Assessment

Epidemiological data are inadequate to determine conclusively the carcinogenic activity of NDMA in humans. Toxicology studies on several species (*e.g.* rats, mice, hamsters, mink, guinea pigs, rabbits, dogs and fish) have been conducted with NDMA at different doses and routes of administration. IARC (1978) concluded there is sufficient evidence for carcinogenicity of NDMA in animals and accordingly IARC classified NDMA as probably carcinogenic in humans (Class 2A). The USEPA classified NDMA as a "probable" human carcinogen (Group B2) (USEPA, 1987).

Of all the animal bioassays conducted on NDMA, the work of Peto and Gray (1984) is generally accepted to be the most appropriate for NDMA risk assessment. The California Department of Health Services (CDHS) (1988) judged the Peto and Gray (1984) work to be the most appropriate for evaluating NDMA potency for several reasons: (i) multiple dose levels (16) were used; (ii) adequate numbers of animals were used (large scale); (iii) a wide range of dose responses, including the lowest dose of 33 µg/L; and (iv) time-to-time data were considered.

In Ontario, an Expert Committee was established in May 1990 to identify potential guideline numbers and associated risk levels for NDMA based on health considerations (OMOE, 1991). During the Ontario guideline development four groups using the same bioassay data from the British Industrial Biological Research Association (BIBRA) (dataset of Peto and Gray, 1984) developed different versions of an acceptable NDMA level for drinking water. Using the same bioassay data, the four risk assessments for NDMA in drinking water, resulted in calculated maximum allowable NDMA concentrations that varied over 5100 fold (**Table 5.1**).

The same raw data from Peto and Gray (1984) were also used by USEPA and CDHS for their human health risk estimates (**Table 5.2**). Interestingly, when developing human health guidelines for NDMA, the OMOE, CDHS and USEPA all derived different risk estimates for NDMA in drinking water but used the same data set.

Table 5.1. Comparison of NDMA risk assessments and the variation in proposed acceptable NDMA concentrations (adapted from OMOE, 1992). Note: BIBRA= British Industrial Biological Research Association.

	MOE/Peto	EPA/Peto/ Waterloo	CanTox/Uniroyal	Health and Welfare Canada
Bioassay	**BIBRA**	**BIBRA**	**BIBRA**	**BIBRA**
Model	Weibull	Weibull	LinearizedMulti-stage	Linear Robust
Rats/TumoursUsed	F only; all	F only; all	Mean of M&F specific types only	M only; all
Hyperplastic Nodules Included?	No	No	No	Yes
Rat Lifespan	3 years	3 years	2 years	2 years
Dose Scaling Factor	6.5	6.5	1	6.5
Water Consumption	1.5 L	2.0 L	1.5 L	1.5 L
Risk Level	1×10^5	1×10^6	1×10^5	1×10^6
Peer Review	Based on EPA	Extensive	None	Inadequate
Proposed [NDMA]	**9 ng/L**	**0.68 ng/L**	**205.6 ng/L**	**0.04 ng/L**

Table 5.2. Comparison of interagency's proposed risk based standards (OMOE, 1992; USEPA, 1987; and CDHS, 2002).

	OMOE	**USEPA**	**CDHS**
***Slope Factor (mg / kg * day)**	**51**	**51**	**16**
Model	**Weibull**	**Weibull**	**Linearized multi-stage**
Acceptable Risk Level	$\mathbf{1 \times 10^{-5}}$	$\mathbf{1 \times 10^{-6}}$	$\mathbf{5 \times 10^{-6}}$
Proposed Risk Based Standard (ng/L)	**9**	**0.7**	**10**

Differences in risk estimates arise from the varied assumptions used in each agency's risk assessment. Clearly, guideline development using the risk assessment process is not entirely objective. One important area where risk assessments are based on subjective policy decisions relates to the interpretation of low dose toxicology data.

Briefly, below the lowest administered dose, a linear extrapolation is applied, which intersects with the origin (**Figure 5.1**). The anchoring of the dose-response curve at the origin is a policy-based decision not a scientific one (Hrudey, 1998). Where to anchor the dose-response curve has major implications in the calculated risk level because risk is calculated as the slope (q^*) × dose. Further, with regards to lifetime cancer risk assessment values and the linearized multistage model, the USEPA (1986) include an important qualifier on risk estimates: "such an estimate, however, does not necessarily give a realistic prediction of the risk. The true value of the risk is unknown, and may be as low as zero. The range of risks, defined by the upper limit given by the chosen model and the lower limit which may be as low as zero, should be explicitly stated." Thus with NDMA a great deal of uncertainty exists when setting regulatory guideline values and this uncertainty must be acknowledged and communicated to those who are required to make risk management decisions.

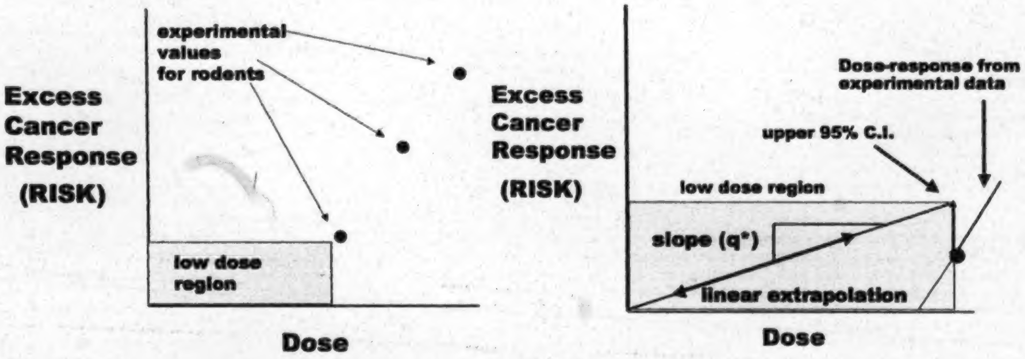
experimental values for rodents
Excess Cancer Response (RISK)
low dose region
Dose
Dose-response from experimental data
Excess Cancer Response (RISK)
upper 95% C.I.
low dose region
slope (q*)
linear extrapolation
Dose

Figure 5.1. Development of low dose cancer risk estimates.
Note: RISK = Slope factor (q*) x Dose.

Risk assessment is intended to be used to inform the risk management process (enHealth, 2002). In the end, risk management involves personal judgment, assumptions and policy-based decisions. Therefore, risk management decisions are not and should not be considered totally objective and free of bias. Risk management is about making tradeoffs and balancing risks, even in the face of limited data and huge uncertainties. This is certainly true in the case for setting drinking water guidelines for NDMA.

5.5 How Many Bladder Cancer Cases Could Theoretically Be Attributed to Drinking Water NDMA Exposures?

As previously mentioned urinary bladder cancer is the cancer outcome most consistently observed in epidemiology studies of long-term exposures to chlorinated drinking water. Estimates of new cases of bladder cancer for 2005 are: 5000 and 63000 for Canada and the United States respectively (CCS, 2005; ACS, 2005). In men, new cases of bladder cancer are estimated to account for 7% (4th overall) of all new diagnosed cancers behind prostate; lung and bronchus; and colon and rectum, (not included are basal and squamous cell skin cancers). Additionally, Canadian men are estimated to have a 1 in 37 lifetime probability of developing bladder cancer, while in American men the lifetime probability is 1 in 28. A female's lifetime probability of developing bladder cancer is lower, 1 in 107 and 1 in 88 for Canadians and Americans respectively.

Epidemiology based population-attributable risk (PAR) estimates for bladder cancer, resulting from long-term exposures to chlorinated DBPs, was estimated to be 14%–16% (King and Marrett, 1996). The USEPA used a PAR range of 2%–17%, based on five epidemiology studies, in economic calculations for the Stage 2 DBP Rule (2003). Based on the widest range of PAR estimates (2%–17%), if a causal link was actually established, approximately 100 to 900 new Canadian and 1300 to 11000 new American bladder cancer cases could be attributed to DBPs. Alternatively, given no exposure, the estimates would reflect numbers of cases that did not occur. It is important to recognize that given the high degree of uncertainty, lower boundary PAR estimates could be as low as zero cases (USEPA, 2003).

Bladder cancer risks from PAR estimates are derived from chlorinated DBPs exposures, typically indexed by THM and HAA concentrations. However, mechanistic evidence for bladder cancer based on drinking water THM and HAA exposures is lacking. *N*-Nitrosamines are more potent carcinogens by 2 to 4 orders of magnitude, compared to THMs based on oral slope factors ($[(mg/kg)/d]^{-1}$), drinking water unit risks $(\mu g/L)^{-1}$ and inhalation unit risks $(\mu g/m^3)^{-1}$ (derived from USEPA Integrated Risk Information System (IRIS) estimates (**Table 5.3**). As used here, "oral slope factor" is defined as an upper-bound estimate of the human cancer risk per mg of agent/kg body weight/day (USEPA, 2005). "Unit risk" is calculated from the slope factor, which is an estimate in terms of either risk per ug/L drinking water, or risk per ug/cu.m air concentration (USEPA, 2005).

Table 5.3. *N*-Nitrosamine and trihalomethane unit risk comparisons based on USEPA Integrated Risk Information System (IRIS) database estimates.

Compound	**Oral Slope Factor $[(mg/kg)/d)]^{-1}$**	**Drinking Water Unit Risk 1×10^{-6} $(\mu g/L)^{-1}$**	**Inhalation Unit Risk 1×10^{-6} $(\mu g/m3)^{-1}$**
		N-Nitrosamines	
NDMA	51	1400	14000
MENA	22	630	ND
DENA	150	4300	43000
DPNA	7	200	ND
NPyr	2.1	61	610
DBNA	5.4	160	1600
		Trihalomethanes	
TCM	NA	NA	23
BDCM	0.062	1.8	ND
DBCM	0.084	2.4	ND
TBM	0.0079	0.23	1.1

Note: NA = Not appropriate, based on USEPA, 2001
ND = No data available

Evaluating NDMA concentrations from food ingestion and inhalation exposures, based on estimates from WHO (2002), and drinking water NDMA concentrations found in Alberta (Charrois et al., 2004), a range of exposure values was derived (**Table 5.4**). Using the range of exposure estimates, a range of lifetime cancer risk estimates were calculated based on the USEPA slope factors and unit risk estimates from **Table 5.3**. Finally, annual cancer risk estimates for Canada and the United States were calculated

(**Table 5.5**) based on a 70 year life expectancy and population estimates of 33 million for Canada and 296 million for the United States.

Comparing the PAR estimates with the theoretical NDMA annual cancer risk estimates two things become clear. Firstly, the upper limit PAR estimates far exceed the annual cancer estimates in spite of being derived from less potent carcinogens. Secondly when exposures from multiple sources are considered, drinking water estimates account for a small proportion of the theoretical number of cases compared to ingestion of foodstuffs. Only when extreme drinking water NDMA concentrations (200 ng/L) are applied to the entire population for a lifetime do annual risk estimates approach calculated cancer risks from food or inhalation. Based on the limited drinking water DBP occurrences of NDMA based on my survey work (Chapter 3), the risk of bladder cancer from NDMA as a DBP appears to be to low.

Table 5.4. Exposures estimates to *N*-nitrosodimethylamine through food, drinking water and inhalation routes.

Range	Food[a] (μg/kg/day)	Drinking Water[b] (ng/L)	Inhalation[c] (μg/m^3)
Min	4.3E-03	2.0E+00	1.7E-03
Max	1.1E-02	2.0E+02	2.4E-01

[a] Based on WHO (2002) data for reasonable worst-case estimates of daily NDMA food intake.
[b] Based on Charrois et al. (2004) Alberta NDMA drinking water concentrions.
[c] Based on WHO (2002) data for reasonable worst-case estimates of daily NDMA inhalation.

Table 5.5. Lifetime cancer risk estimates from exposures to *N*-nitrosodimethylamine through food, drinking water and inhalation routes. Predictions based on lifetime estimates from Table 5.4.

Range	Food	Drinking Water	Inhalation
	Predicted Canadian Cancer Cases		
Min	2.2E-04	2.8E-06	2.4E-05
Max	5.6E-04	2.8E-04	3.4E-03

Table 5.6. Annual cases of cancer predicted from exposures to *N*-nitrosodimethylamine through food, drinking water and inhalation routes. Predictions based on lifetime estimates from Table 5.5.

Range	Food	Drinking Water	Inhalation
	Predicted Canadian Cancer Cases		
Min	100	2	10
Max	270	130	1600
	Predicted American Cancer Cases		
Min	900	10	100
Max	2400	1200	14000

5.6 Conclusions

N-Nitrosamines are an unregulated class of DBPs, which are 2 to 4 orders of magnitude more potent than regulated halogenated DBP species, specifically THMs and HAAs. The United States Environmental Protection Agency's (USEPA) weight-of-evidence characterization for chloroform, a regulated THM and often the most abundant DBP species, concludes that chloroform is likely to be carcinogenic under high exposure conditions (leading to cytotoxicity and regenerative hyperplasia). At relatively low exposure concentrations, such as those typical in drinking water, chloroform is not recognized as a carcinogen. Thus, a reevaluation of the contributions of specific DBPs to health risk is necessary. Specifically, researchers need to consider whether regulated DBPs have the biological plausibility to cause the adverse health outcomes we are trying to avoid.

More *N*-nitrosamine occurrence data from Canadian utilities with a variety of treatment practices, particularly outside Ontario (which has been the only focus in Canada thus far), would be useful in determining whether *N*-nitrosamines in Canadian drinking waters are a conceivable public health concern. A holistic human health risk assessment, however, requires perspective not only on drinking water contributions of *N*-nitrosamines but also the relative individual daily intake from all sources, including food, consumer products and endogenous production. Calculations based on NDMA as a drinking water DBP suggest: i) drinking water is typically a minor source of NDMA exposure and ii) based on limited occurrence data, the risk of developing cancer from lifetime NDMA drinking water exposures appears to be low.

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Chapter Six

Conclusions and Synthesis

6.1 Introduction

Drinking water providers ensure safety from pathogenic organisms through the effective use of disinfectants. Disinfection of drinking water is not entirely risk-free, however, the risks associated with no treatment are certainly much greater. Since the 1974 discovery of trihalomethanes as disinfection by-products (DBPs) in drinking water, the regulatory and public health focus has been primarily directed at halogenated DBPs, even though it is well established that chlorination and chloramination also produce non-halogenated DBPs. Specific halogenated DBPs that could reasonably explain the correlation of some adverse health outcomes with consumption of disinfected drinking water in a number of epidemiologic studies have yet to be identified. Early in my thesis program *N*-nitrosodimethylamine (NDMA), began emerging as a non-halogenated DBP of interest. I became interested in NDMA because unlike traditional DBPs such as THMs and HAAs, *N*-nitrosamines have been shown to be carcinogenic in almost every tissue they were tested in. Given public health concerns for possible correlations between bladder cancer and consumption of chlorinated drinking water, further consideration of *N*-nitrosamines as DBPs was warranted.

Several aspects of the research conducted in support of this dissertation have contributed to the advancement of knowledge in the area of drinking water quality. My thesis investigation of *N*-nitrosamines as drinking water DBPs can be divided into four interrelated phases that include: (1) developing an analytical method capable of extracting and detecting a range of *N*-nitrosamines at relevant drinking water concentrations (ng/L) (**Chapter 2**); improving the original analytical method's sample processing capacity and applying the technique as part of a *N*-nitrosamine occurrence survey in Alberta (20 utilities) (**Chapter 3**); comparing the NDMA formation potentials from full-scale chloramination processes and exploring the influence of Cl_2:NH_3-N ratios on the production of NDMA as an attempt to identify potential treatment process options for drinking water utilities experiencing elevated NDMA concentrations (**Chapter 4**); and finally evaluating lifetime cancer-risks from NDMA drinking water exposures and providing a risk management context from which to base future drinking water quality decisions (**Chapter 5**). Additionally, I participated in a blinded, international, multi-centre (14 laboratories) analytical round-robin study that compared *N*-nitrosamine data from within a variety of matrices (**Appendix A**). What follows are summaries of the key results and advances from **Chapters 2–5**, with a synthesis of results based on my experimental findings as well as recommendations for reducing City A's elevated NDMA concentrations, and finally comments on future DBP research directions.

6.2 Advancements in Knowledge

6.2.1 Chapter 2: Detecting *N*-nitrosamines in drinking water at ng/L levels

N-Nitrosamines, which can occur at ultra-low concentrations (ng/L), are hydrophilic, polar, compounds making them challenging to extract and to detect in water. Furthermore, many *N*-nitrosamines are "probable" human carcinogens. Until recently the only reliable method for accurate ng/L level detection of *N*-nitrosamines in water was to use costly high-resolution mass spectrometry instrumentation. With increased utility monitoring and continued research into *N*-nitrosamine formation reactions, improvements in analytical methods are necessary. My development of a new analytical method, capable of detecting eight *N*-nitrosamines at relevant drinking water concentrations was highly significant because it utilized lower cost instrumentation, namely a bench top GC/MS. To overcome the analytical challenges mentioned above, a dual stage solid-phase extraction (SPE) technique was combined with GC/MS instrumentation, using ammonia reagent gas in positive chemical ionization (PCI) mode.

The first phase of my thesis research resulted in the development of a selective, sensitive (detection limits of 0.4 ng/L–1.6 ng/L) and affordable bench-top GC/MS analytical method for detecting *N*-nitrosamines in drinking water. Applying my extraction method to authentic drinking water samples, from two Alberta locations with dissolved organic carbon concentrations of 9 mg/L, we were able to detect *N*-nitrosodimethylamine (2–180 ng/L) as well as *N*-nitrosopyrrolidine (2–4 ng/L) and *N*-nitrosomorpholine (1 ng/L), two *N*-nitrosamines not reported in drinking water before. Concentrations of NDMA approaching 200 ng/L in treated drinking water were among the highest ever reported, without anthropogenic point source contamination. Analytes were quantified using both isotope dilution / surrogate standard and internal standard procedures. Quality control samples indicated the method was capable of achieving high recoveries of standards and analytes, which made quantification of several *N*-nitrosamines with an internal standard practical.

6.2.2 Chapter 3: Surveying Alberta drinking water for *N*-nitrosamines

A major limitation to the analytical method described in **Chapter 2** was the fact that only two samples could be extracted per day. Any practical application of the technique in a commercial, regulatory, utility or academic laboratory would require modifications allowing for increased sample processing. In order to achieve higher sample throughputs, I modified a commercially available solid-phase extraction manifold, which allowed increased sample extraction capacity of up to 10 samples per day. Not only could additional samples be extracted but the manifold also allowed for an improved capacity for quality control samples (blanks, laboratory fortified blanks, and replicates samples) to be included with each batch of samples. Use of a high capacity SPE method will result in lower costs, shorter processing times, and higher sample throughput.

I took part in an analytical round-robin, with 13 other laboratories representing commercial, utility, regulatory and academic analysis sectors, using the modified SPE manifold technique coupled with GC/MS ammonia PCI (**Chapter 3**). The round-robin was part of a project funded by the WateReuse Foundation (WRF; Arlington, VA) entitled "*Alternative Methods for the Analysis of NDMA and Other Nitrosamines in Water and Wastewater*". Results from several different sample matrices (including wastewater, recycled water, surface water and groundwater samples) indicated my method was highly comparable to the two gold standard laboratories, one of which used high-resolution mass spectrometry. The round-robin provided me an opportunity to compare and validate my analytical method (**Appendix A**), an achievement not undertaken in most thesis projects.

After method validation, I successfully implemented the manifold technique in a survey of twenty Alberta municipal drinking-water distribution systems, examining the occurrence of *N*-nitrosodimethylamine (NDMA) and seven other *N*-nitrosamine species (summer 2004). I documented the presence of NDMA (up to 100 ng/L; median < 5 ng/L) as well as two other *N*-nitrosamines (*N*-nitrosopyrrolidine and *N*-nitrosomorpholine) in

Alberta drinking water supplies. Overall NDMA was detected in 30% of the distribution systems sampled, which served a majority of Alberta's population and included all but one of Alberta's chloraminating facilities. Only two water systems were identified as having NDMA concentrations above Ontario's Drinking Water Quality Objective (ODWQO) of 9 ng/L, which was encouraging from a provincial perspective. To my knowledge, this was the first Canadian survey of *N*-nitrosamines outside Ontario.

A statistical evaluation of the existence of trends between routine water quality parameters (pH, DOC, SUVA, NH3-N, NO_3^-, TKN, and organic nitrogen) and NDMA concentrations was also conducted. Potential trends between NDMA and SUVA or DOC were identified, though neither was statistically significant at p=0.05 (p=0.07 and 0.09 respectively). A more comprehensive dataset containing *N*-nitrosamine occurrence data and routine water parameters would be helpful to better evaluate this apparent trend. Additionally, **Chapter 3** summarized publicly available NDMA data from Ontario (1994–2002) and California (1999) influent, effluent and distribution samples. Results indicated NDMA occurrences were typically less than 2 ng/L for most utilities, however, there was a subset of facilities that continually experienced elevated NDMA concentrations.

My work in **Chapter 3** advanced the state of water quality knowledge through the development of a SPE method capable of producing analytical results comparable to but less costly than methods using high-resolution mass spectrometry in addition to having the capacity to extract 10 samples simultaneously. The method is simple and robust and could be used by other water quality laboratories. Application of the method for surveying Alberta public drinking water distribution systems provided much needed Canadian *N*-nitrosamine occurrence data.

6.2.3 Chapters 2 and 3: Evaluating a long term data set

Results from **Chapter 2** combined with the Alberta *N*-nitrosamine survey data (**Chapter 3**) and additional periodic monitoring of Alberta drinking waters, highlighted one Alberta location (City A) with exceptionally high NDMA levels in finished drinking water. Temporal trends from comprehensive sampling in City A were based on samples collected between July 2003 and June 2005. The historical dataset I generated not only documents exceptionally high NDMA concentrations in drinking water but it also contains multiple distribution system sampling locations. Concentrations of NDMA were found to be variable over time and I observed increasing NDMA levels with increasing time in the distribution system. The issue of distribution residence time has important implications for human exposure studies because earlier NDMA estimates, taken only at water treatment plants, may under estimate actual population exposures.

6.2.4 Chapter 4: Evaluating factors related to *N*-nitrosamine formation

Having identified a unique location (City A), in terms of NDMA occurrence, prompted further investigations into factors that may influence the formation of NDMA. Bench-scale experiments were part of an evaluation of two chloraminating Alberta water treatment plants. The two treatment plants were selected for further investigation because they both: used surface waters with high DOC concentrations, used the same cationic polymer (poly-DADMAC), chloraminated; and had strikingly different NDMA distribution system concentrations. From evaluating these two treatment plants, I concluded that high poly-DADMAC doses were not a sufficient condition to produce elevated NDMA concentrations and that a period of free-chlorine contact prior to ammonia addition can reduce the formation potential of NDMA. Bench-scale chloramination experiments were conducted using raw water as well as partially-treated water (collected prior to disinfection, but after coagulation, flocculation, sedimentation, and filtration) from the two treatment plants. Maximum NDMA formation occurred in the sub-breakpoint region of the disinfection curves. Additionally, bench-scale chloramination experiments that had a period of free-chlorine contact (up to 2 hours) before ammonia addition, resulted in reductions of NDMA up to 93% of values without any free-chlorine contact time.

A trend among North American drinking water treatment plants is to switch from chlorination-only disinfection to alternative disinfection methods such as chloramination as a way to reduce regulated DBP levels trihalomethanes (THMs) (Canada and US) and haloacetic acids (HAAs) (US Only). Ironically, in an attempt to reduce regulated DBP concentrations, alternative disinfection processes such as chloramination have been found to generate unregulated DBPs such as *N*-nitrosamines that may be of equal or greater health concern compared to THMs and HAAs. Results from **Chapter 4** provide valuable insights for evaluating risk-tradeoffs when considering incorporating chloramination processes. Additionally, data from **Chapter 4** highlight treatment options for water treatment plants experiencing elevated NDMA concentrations. Finally, results from

Chapter 4 indicate there can be contributions of NDMA precursors from multiple sources. Chloramination of raw water NOM alone (under realistic disinfection conditions) can generate NDMA in addition to NDMA formed by disinfecting water containing cationic polymers (poly-DADMAC).

6.2.5 Chapter 5: Managing risks from *N*-nitrosamines in drinking water

Since discovering chloroform in drinking water, the water industry has been preoccupied with halogenated compounds resulting from disinfection practices. The two main classes of disinfection by-products (DBPs), trihalomethanes (THMs) and haloacetic acids (HAAs) have been the focus the of health and water quality researchers. From a toxicology perspective neither THMs nor HAAs are sufficiently potent or mechanistically plausible carcinogens to account for the adverse health effects estimated in some epidemiology cancer studies.

The DBP class evaluated in this thesis was *N*-nitrosamines, which warranted further investigation because of their detection in drinking water and wastewater as well as the fact that *N*-nitroso compounds, as a class, have produced tumors in every vital tissue tested. *N*-nitrosodimethylamine (NDMA) is a carcinogen that has received a lot of attention within the past fifteen years as a DBP, first in Ontario, then in California and finally in Alberta. Typically NDMA concentrations in drinking water are in the sub-ng/L to 10 ng/L range (parts-per-trillion). Work conducted in **Chapters 2 and 3** indicated there were a limited number of Alberta locations in excess of Ontario's Drink Water Quality Objective (ODWQO) of 9 ng/L. One location in particular (City A) had measured NDMA concentrations near 200 ng/L.

During the NDMA drinking water regulatory development process in Ontario, numerous assumptions were used in a variety of different risk assessments. When combined, the varied approaches in risk assessments resulted in the calculation of a maximum acceptable NDMA dose that ranged over more than 5100 fold. Interestingly, all the risk assessments used the same experimental toxicology data. Results from Ontario's regulatory procedure for developing an NDMA drinking water guideline showcase the subjective nature of a process often considered objective by the public. Given recent public and research interest in NDMA as a DBP as well as having two jurisdictions with NDMA guidelines and no national standards, **Chapter 5** explored the question of, "are we really managing human health risks?"

The significance of *N*-nitrosamine exposures from drinking water needs to be put into a larger context for human health assessments, to evaluate fully public health protection. In the past multiple routes of exposure to NDMA have not fully considered in NDMA drinking water guidelines. It is well established that NDMA and NDMA precursors are commonly found in foodstuff and consumer products.

Bladder cancer, the cancer widely acknowledged as the most plausible cancer (if any) to develop from long-term exposures to chlorinated drinking waters, was considered in a lifetime cancer risk assessment of exposures to NDMA. Results suggest that NDMA drinking water exposures represent a minor component of tot al NDMA exposure. Thus, any when considering regulating NDMA in drinking water, it will be important to have a comprehensive exposure evaluation, to truly protect the public's health.

Chapter 5 makes advances in the area of drinking water quality because considerations are given to multiple routes of NDMA exposure, something that was not done in current guidelines. Additionally, lifetime cancer risks were put into a national context to help judge the relative importance of drinking water NDMA.

6.3 Recommendations for City A and Practical Implications

Early in the thesis project, City A drinking water was identified as having elevated NDMA levels. Concentrations of NDMA in the 200 ng/L range were closer in magnitude to treated wastewater values than to NDMA levels reported in the limited number of existing drinking water occurrence surveys. Additionally, two previously unidentified *N*-nitrosamines, NMor and NPyr were identified in City A's drinking water. An independent water utility laboratory confirmed our original *N*-nitrosamine concentrations (July 2003), establishing a strong basis from which to investigate the elevated NDMA occurrences.

City A officials were informed of the *N*-nitrosamine results and supplementary sampling at the original sites as well as additional locations was under taken in September of 2003. A second round of sampling found lower NDMA drinking water concentrations but the levels were still much higher compared to reports from other jurisdictions. In addition to City A officials, provincial authorities from Alberta Environment, Alberta Health and Wellness as well as the Medical Officer of Health responsible for City A were informed of the *N*-nitrosamine results by way of an informational meeting. It was agreed that additional monitoring of City A's drinking water would be conducted.

Neither a Canadian drinking water guideline nor an Alberta potable water quality standard for NDMA existed, so City A was not at any time in violation of any conditions of their operating license. The only established Canadian NDMA value was Ontario's Drinking-Water Quality Standard (ODWQS) of 9 ng/L, which was used as a bench mark for comparing measured NDMA concentrations. Additionally, California had established a Notification Level (NL) for NDMA of 10 ng/L.

The City Engineer and staff from City A's water treatment plant were committed to understanding more about NDMA formation within their system as well as identifying potential process options that may reduce NDMA formation. The openness and cooperative nature of all water treatment personnel from City A was a major determinant that facilitated the significant investigation of treatment plant processes as well as the research into source and partially-treated waters.

In terms of *N*-nitrosamine control, based on experimental bench-scale testing results from **Chapter 4,** I recommend City A consider modifications to their water treatment process that would allow for an extended free-chlorine contact time (perhaps up to a few hours) prior to ammonia addition. An evaluation, bench-scale and possibly pilot-scale, of the impact that additional free-chlorine contact will have on regulated DBPs, namely THM concentrations, would also be necessary. Extended free-chlorine contact time would have the benefit of providing additional CT credits for pathogen inactivation, under Alberta's proposed "Standards and Guidelines for Municipal Waterworks, Wastewater and Storm Drainage Systems" (AENV, 2005).

Bench-scale testing would also provide an opportunity to evaluate different cationic polymers, for aiding coagulation. Although the diallyldimethylammonium chloride (poly-DADMAC) based cationic polymers used in City A are known to contain NDMA precursors, experiments in **Chapter 4** demonstrated that poly-DADMAC dose alone is not the sole determinant of NDMA formation. Selection and evaluation of alternative cationic polymers could allow City A to achieve their necessary performance targets for coagulation and filtration while simultaneously removing a known source of NDMA precursors. Finally, results from **Chapter 4** established that, at least for City A, NDMA precursors are also present in the source water. Improved removal of natural organic material from City A's source water prior to disinfection may also afford opportunities to decrease NDMA formation potentials. Enhanced organic matter removal could be achieved a number of ways including incorporation of membranes (microfiltration, ultrafiltration, nanofiltration, or reverse osmosis) or activated carbon contact chambers. Increased initial source water treatment would also improve other performance parameters critical for disinfection such as reduced turbidity and particle counts.

Prior to incorporating any full-scale modifications to City A's treatment plant, consideration of the feasibility (economic and engineering) of incorporating the proposed recommendations is required. A detailed facility assessment was not within the scope of this project. Inclusion of any process changes ought to be made only after establishing treatment priorities and evaluating potential tradeoffs between perceived risks and added benefits.

6.4 Future Research

Source water and treatment plant processes are location dependent. As alternative disinfection processes continue to evolve utility managers considering process changes need to base their decisions on the best data available. This may involve conducting site-specific bench- or pilot-scale testing prior to initiating full-scale changes. In order to have the best available data from which to make decisions, additional DBP research must continue. Advancements achieved through my thesis research have also highlighted additional areas in need of future DBP research.

During the course of my thesis project, attempts to standardize *N*-nitrosamine analysis were made, most notably the completion of EPA Method 521, *Determination of Nitrosamines in Drinking Water by Solid Phase Extraction and Capillary Column Gas Chromatography with Large Volume Injection and Chemical Ionization Tandem Mass Spectrometry (MS/MS)*. Application of Method 521 as well as other analytical methods is required to generate an improved occurrence dataset for NDMA and other *N*-nitrosamines in drinking water. Identifying additional locations with elevated NDMA levels such as those measured in City A (**Chapter 2**) need to be determined. Even though data compiled to date (**Chapter 3**) suggest median NDMA drinking water concentrations are low, generally < 2 ng/L, the *N*-nitrosamine occurrence dataset is not comprehensive.

Improved occurrence information will give regulators a stronger basis from which to consider any potential future regulatory decisions.

Continued investigation into DBP formation mechanisms will aid in developing water treatment processes that minimize the production of *N*-nitrosamines and other alternative DBPs in drinking water. However, when evaluating human health risks posed by *N*-nitrosamines, a more comprehensive assessment of exposure is required. A reevaluation of NDMA and NDMA precursors in foodstuff and consumer products is necessary, as many manufacturing process changes have occurred during the last few decades. Additionally, future risk assessments conducted by drinking water regulators ought to incorporate complete exposure scenarios from all routes of exposure. Based on limited data, drinking water generally appears to represent only a minor source of *N*-nitrosamine exposure. Thus any potential *N*-nitrosamine regulation should be founded in an interdisciplinary approach that involves regulatory stakeholders from multiple governmental departments (*e.g.* environment and health). Any *N*-nitrosamine regulation should consider exposures from drinking water as well as food and consumer products.

The need to evaluate risk trade-offs between regulated DBPs and alternative DBPs, such as *N*-nitrosamines will continue. In the face of uncertainty, decisions must be made based on the best available evidence. For examples, utilities considering switching from free chlorine- to chloramine-based disinfection processes ought to consider not only potential long-term health implications of a shifting suite of DBP species but also short-term health consequences that could result (*e.g.* elevated exposure to lead in drinking water).

6.5 Conclusions

Issues related to DBPs have now been studied for over thirty years and it is unlikely that the near or even distant future will see a resolution to DBP-related uncertainties faced today. Unquestionably, challenges to producing safe drinking water and complexities involved in delivering drinking water through distribution systems will continue. Small changes in one area of the treatment process can have dramatic consequences for another unanticipated aspect of the system. Drinking water systems are ever changing. Modifications, even to routine procedures, require as fully of an informed decision as possible. Water treatment operators and managers must be continually preparing for unforeseen changes and be ready to evaluate new information as rigourously and objectively as possible when a new challenge arises. Consumers on the other hand, must stop undervaluing the cost of drinking water as well as start appreciating the societal benefits accrued to the public's health resulting from the provision safe drinking water.

A new DBP research agenda ought to be guided by outcomes from relevant water quality, toxicology and epidemiology studies. Research priorities must be refocused to address directly key data gaps associated with the most relevant long-term endpoint in DBP related epidemiology studies, bladder cancer. Analytical identification of a single DBP or mixture of drinking water DBPs capable of producing bladder cancer would decrease current uncertainties and advance public health protection. Until a causative agent(s) is (are) identified, a precautionary approach for safe drinking water production would be first to establish control over known hazards such as microbial pathogens and only after, to pursue the most plausible research questions related to DBPs.

Drinking water quality affects everyone and delivering poor quality water can directly and quickly have negative impacts on a population. Therefore, I submit that through evidence-based decision-making, the ultimate goal of any drinking water provider ought to be the continual production and delivery of safe water, which ultimately will ensure public health protection.

6.6 References

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Appendix A

N-Nitrosamine Method Validation: Participation in a Blinded, Multi-Centre, Analytical Round-Robin

A.1 Introduction

Between February and April 2004 I took part in a blinded, multi-centre, analytical round-robin, analyzing *N*-nitrosamines in water and wastewaters samples. Participating in the analytical round-robin allowed an opportunity to validate the solid-phase extraction (SPE) method coupled with GC/MS ammonia positive chemical ionization (PCI) used throughout this thesis. For the purposes of this chapter, the term "validation" is used as described by Taylor (1987), "the process by which a sample, measurement method, or a piece of data is deemed useful for a specified purpose" (p.253). Method validation involved value judgments concerning performance parameters of the method, which were compared to requirements for the analytical data. From the standpoint of my thesis work the specified purpose of validation was to confirm the accurate and reproducible performance, of my SPE GC/MS ammonia PCI method, for quantifying eight *N*-nitrosamines (**Table A.1**) at relevant drinking water concentrations (low ng/L) as well as within complex matrices such as wastewater. In the context of this thesis, method validation does not imply that multiple independent laboratories tested the analytical procedure or that the process involved a formal framework such as might be required in a regulatory submission (*e.g.* formal pharmaceutical method guidelines established by the United States Food and Drug Administration (FDA) or the United States Pharmacopeia (USP)).

The California Department of Health Services (CDHS, 2005), however, established a series of analytical requirements, which if met would be considered acceptable to CDHS for the analysis of *N*-nitrosamines in water. A partial list of CDHS (2005) requirements met by the University of Alberta method includes:

Table A.1. Target *N*-nitrosamines.

Compound	Abbreviation
N-Nitrosodimethylamine	NDMA
N-Nitrosomethylethylamine	MENA
N-Nitrosodiethylamine	DENA
N-Nitrosopyrrolidine	NPyr
N-Nitrosopiperidine	NPip
N-Nitrosomorpholine	NMor
N-Nitroso-*n*-dipropylamine	DPNA
N-Nitrosodi-*n*-butylamine	DBNA
Isotopes	
[6-^{2}H] *N*-Nitrosodimethylamine	NDMA-*d6*
[2-^{15}N] *N*-Nitrosodiethylamine	DENA-*N2*
[14-^{2}H] *N*-Nitroso-*n*-dipropylamine	DPNA-*d14*

- a calibration curve consisting of at least five standards from approximately 1 ng/L to 100 ng/L range, bracketing the concentrations in the original sample, must be available. Mean response factors (RF) and standard deviations (SD) are to be calculated from the calibration standards. Relative standard deviations (RSD) must be within 20%;
- both precision and accuracy of the analytical process must be demonstrated for each batch of samples by the analysis of matrix spike and matrix spike duplicates (MS/MSD) for a given reporting level; and
- precision as measured by the RSD should be within 20%. Accuracy as measured by % recovery should be 70%–130% for laboratory fortified blanks (LFB) using MS/MSD.

Note: it was not the objective of the round-robin to meet all the analytical requirements setout by CDHS, however, the established criteria were useful for comparative purposes.

Fourteen laboratories (9 in the United States and 5 in Canada) representing commercial, utility, regulatory and academic sectors (including the University of Alberta's Environmental Health Sciences Laboratory), were invited to take part in the round-robin portion of a larger project sponsored by the WateReuse Foundation (WRF; Arlington, VA) entitled "*Alternative Methods for the Analysis of NDMA and Other Nitrosamines in Water and Wastewater*". The primary objective the WRF project was, "to develop alternative and reproducible analytical techniques for measuring NDMA and other nitrosamines in various matrices, including wastewater, recycled water, surface water and groundwater samples".

A variety of extraction methods coupled with GC/MS quantification were evaluated as part of the analytical round-robin testing including:

- liquid-liquid extraction (LLE), manual or continuous;
- micro liquid-liquid extraction (MLLE);
- free Ambersorb solid-phase extraction (Amb SPE);
- cartridge solid-phase extraction (Amb-Envi CSPE); and
- modified cartridge solid-phase extraction (Mod CSPE[1]).

Participating laboratories also used a range of mass spectral instrumentation including: high-resolution magnetic sector (HRMS), low-resolution electron impact ionization (EI-MS) as well as chemical ionization (CI-MS) with quadrupole or ion trap. Chemical ionization reagent gases included: methanol, acetonitrile and ammonia.

Substantial efforts from participants in the WRF project resulted in a significant compilation of *N*-nitrosamine methods as well as a thorough and systematic review of analytical method performances. Readers seeking more detailed information regarding outcomes from the project should consult the WRF report (Cheng et al., 2005).

A.2 Materials and Methods

A.2.1 Composition of round-robin samples

Four different source waters were included in the round-robin evaluation. Sample waters represented a range of matrices:

1. chloraminated potable water;
2. reverse osmosis (RO) effluent from a chlorinated reclamation plant;
3. secondary treated wastewater effluent (2°); and
4. chlorinated tertiary treated wastewater effluent (3°).

N-Nitrosamine concentrations were expected to range from low ng/L to low µg/L levels, however, because the above samples were authentic and uncharacterized, a "true" concentration was not known. However, in order to evaluate method accuracies, known concentrations of a *N*-nitrosamine mixture were spiked into: i) a chloraminated potable water sample and ii) a 2° wastewater effluent sample, creating two additional samples. In total, six samples (**Table A.2**) were prepared and shipped to all participating laboratories. Additional details regarding the organization and execution of the round-robin evaluation can be found in Cheng et al. (2004 and 2005).

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[1] As used in the WateReuse Foundation Report (Cheng et al. 2005), "Mod CSPE" refers to the method used by the University of Alberta's Environmental Health Sciences laboratory and described in **Chapter 3**.

Table A.2. Characterization of round-robin samples[a].

Sample ID	Source	pH	Turbidity (NTU)	Conductivity (µmho/cm)	Total Cl2 (mg/L)	NA Spike[b] (ng/L)
RR-01	Chloraminated potable water	8.3	0.3	420	2.8	0
RR-02	RR-01-Spiked	8.0	0.1	400	2.8	13
RR-03	RO Effluent	4.7	0.1	65	0.05	0
RR-04	2° Effluent	7.4	1.1	820	0.04	0
RR-05	RR-04-Spiked	7.3	0.2	840	0.04	380
RR-06	3° Effluent	7.4	0.4	910	3.6	0

[a] Adapted from Cheng et al., 2005.
[b] NA = *N*-nitrosamine, see **Table A.1** for a list of the eight spiked *N*-nitrosamines.

Three weeks prior to receiving round-robin samples, two common standard solutions for all laboratories were shipped. A mixed *N*-nitrosamine solution, containing the eight analytes of interest as well as an isotopic standard mixture were sent to participating laboratories via overnight courier. The isotopic standard solution was pre-mixed and contained NDMA-*d6*, DENA-*N2*, and DPNA-*d14*. Since the isotopic standards were received as a mixture, a single standard could not selected as a surrogate standard to monitor extraction efficiency.

A.2.2 University of Alberta

The extraction method used to analyze the round-robin samples was identical to the method described in **Chapter 3** of this thesis, which was based on the original SPE method detailed in **Chapter 2**. Overcoming limited sample throughput was the major challenge facing the original SPE method, which was capable of extracting only 2 samples per day. **Chapter 3** details a scaled-up extraction method, accomplished by incorporating a commercially available SPE manifold. The higher-throughput method was capable of processing 10 samples per day. Not only could more samples be processed but equally important was an improved capacity to include more quality control samples (*e.g.* method blanks, laboratory fortified blanks, and replicates of authentic samples).

After extraction, *N*-nitrosamines were chromatographically separated using an Agilent Technologies (Palo Alto, CA) 6890N gas chromatograph coupled with a 5973 mass selective detector (MSD), operating in positive chemical ionization (PCI) mode, with ammonia as the reagent gas. Details of the GC/MS PCI ammonia method can be found in **Chapter 2** of this thesis. In **Chapter 2**, two procedures for quantifying eight *N*-nitrosamines were described: i) isotope dilution / surrogate standard (ID/SS) and ii) internal standard (IS). For the round-robin competition, organizers requested we use isotope dilution as our method of quantification.

A.2.3 Gold Standard (GS) laboratories

While thirteen other laboratories participated in the round-robin, a comparison of analytical results with only two of the laboratories will be presented here. The two laboratories used for comparison will be referred to as: Gold Standard laboratories one and two (GS1 and GS2). The two gold standard facilities were the primary laboratories involved in organization and execution of the WRF project. Both GS1 and GS2 have extensive practical experience analyzing *N*-nitrosamines in water samples. GS2 was the laboratory responsible for making and shipping the primary *N*-nitrosamine calibration solutions to all participants in the round-robin.

Several methods were evaluated in the round-robin. Of interest for this chapter, was the GS1 method, which used the free Ambersorb 572 solid-phase extraction technique in combination with GC/MS/MS (ion trap) PCI methanol quantification. Furthermore, GS2 results were obtained using the cartridge solid-phase extraction method (combination of Envi-carb and Ambersorb 572) coupled with quantification using GC/HRMS.

A.3 Results and Discussion

During the round-robin analytical evaluation, all *N*-nitrosamines quantified by our isotope dilution method, were based on three different *N*-nitrosamine isotope standards (**Table A.3**). Results from our method compared very well with the two gold standard laboratories. For all eight *N*-nitrosamine species our results were within ± 15%

difference for all samples, except two (**Tables A.4–A.9**). One exception of the percent difference exceeding ± 15% was for MENA in sample RR-02 (**Table A.5**). Sample RR-02 was spiked with a known concentration of *N*-nitrosamines (12.7 ng/L). The percent difference between the University of Alberta analysis and the average of the gold standard laboratories was 27%. However, because RR-02 had a known concentration, a comparison can be made with the known spiked concentration. The University of Alberta result was actually more accurate (105% of the known value) for MENA compared to the combined gold standard result (84% of the known value). The second exception where percent difference exceeded ± 15% was for NMor in the RR-03 sample (**Table A.6**). Our method result was 3 ng/L for NMor, while GS2 detected 2.3 ng/L and GS1 reported < 2ng/L.

In terms of low-level accuracy performance (Δ Sample RR-02 minus RR-01) for the University of Alberta method, an average accuracy for all *N*-nitrosamine species was 102 ± 11%, ranging from 88% for NPip to 125% for NMor (**Table A.10**). Similar results were obtained by GS1 and GS2, which overestimated NMor by 148% and 127% and under estimated NPip by 80% and 90% respectively. With respect to high-level accuracy performance (Δ Sample RR-05 minus RR-04), the University of Alberta method obtained an average accuracy for all *N*-nitrosamine species of 95 ± 4%, ranging from 90% for DPNA to 101% for MENA (**Table A.11**). The range of high-level accuracies for the University of Alberta method was 11%, while for GS1 and GS2 the range was 37% and 33% respectively.

As mentioned previously, the California Department of Health Services (CDHS) developed guidelines for evaluating acceptability of *N*-nitrosamine analytical method performance (CDHS, 2005). Method accuracy as measured by % recovery should be 70%–130 %. The University of Alberta average method accuracy for all *N*-nitrosamines was ± 15%. The CDHS suggests precision, as measured by relative standard deviation (RSD), should be within ± 20%. The precision requirement was met using DPNA-*d14* for isotope dilution in all samples. Exceedance of the precision guideline occurred once for each of: i)NDMA-*d6* in the case of NPip RR-05 (22% RSD) and ii) DENA-*N2* in the case of NMor RR-01 (24% RSD).

Overall, the University of Alberta results were comparable to the gold standard laboratories. Cheng et al. (2004) described the University of Alberta method as "promising" and "strongly recommend" additional testing to verify the accuracy and reproducibility of the method.

Note: All *N*-nitrosamine data in **Tables A.3–A.11** are presented only as raw values and are not reported with an appropriate number of significant figures for use. Based on the precision and accuracy of the *N*-nitrosamine analytical method used throughout this thesis, no more than two significant figures are warranted when reporting *N*-nitrosamine values.

Table A.3. Overall results for University of Alberta analysis of round-robin samples. Quantification based on isotope dilution of three *N*-nitrosamine isotope standards.

Sample		Conc'n (ng/L)		Conc'n (ng/L)		Conc'n (ng/L)	
		NDMA-*d6*		DENA-*N2*		DPro-*d14*	
		Ave (n=3)	RSD (%)	Ave (n=3)	RSD (%)	Ave (n=3)	RSD (%)
RR-01	NDMA	2.5	1.0	4.1	1.1	4.1	0.94
	NMor	Not Quantifiable using NDMA-d6		0.8	23.6	0.7	17.33
		Conc'n (ng/L)		**Conc'n (ng/L)**		**Conc'n (ng/L)**	
		NDMA-*d6*		DENA-*N2*		DPro-*d14*	
		Ave (n=3)	RSD (%)	Ave (n=3)	RSD (%)	Ave (n=3)	RSD (%)
RR-02	NDMA	13.9	13.7	16.3	1.6	16.9	3.0
	MENA	10.9	12.3	13.0	2.6	13.4	1.9
	DENA	10.5	13.5	12.5	1.9	13.0	2.0
	DPNA	9.5	12.1	11.5	3.2	11.9	1.2
	NMor	13.8	11.9	16.1	2.8	16.7	2.4
	NPyr	10.2	12.2	12.2	2.7	12.6	2.7
	NPip	9.0	11.5	10.8	3.3	11.2	1.8
	DBNA	10.2	11.8	12.2	3.1	12.6	3.0
		Conc'n (ng/L)		**Conc'n (ng/L)**		**Conc'n (ng/L)**	
RR-03		NDMA-*d6*		DENA-*N2*		DPro-*d14*	
		Ave (n=3)	RSD (%)	Ave (n=3)	RSD (%)	Ave (n=3)	RSD (%)
	NDMA	11.6	10.9	13.9	7.7	14.1	7.9
	NMor	1.8	20.6	3.0	3.8	3.0	4.6
		Conc'n (ng/L)		**Conc'n (ng/L)**		**Conc'n (ng/L)**	
RR-04		NDMA-*d6*		DENA-*N2*		DPro-*d14*	
		Ave (n=3)	RSD (%)	Ave (n=3)	RSD (%)	Ave (n=3)	RSD (%)
	NDMA	165	9.4	168	19.5	163	20.2
		Conc'n (ng/L)		**Conc'n (ng/L)**		**Conc'n (ng/L)**	
RR-05		NDMA-*d6*		DENA-*N2*		DPro-*d14*	
		Ave (n=3)	RSD (%)	Ave (n=3)	RSD (%)	Ave (n=3)	RSD (%)
	NDMA	539	12.2	537	1.7	537	1.7
	MENA	378	20.3	377	6.7	378	5.9
	DENA	361	18.0	361	4.6	362	3.8
	DPNA	336	19.8	336	6.6	337	5.7
	NMor	352	20.4	352	6.6	353	5.9
	NPyr	369	19.3	368	5.6	369	5.1
	NPip	341	21.9	341	8.3	342	7.6
	DBNA	344	19.3	344	6.7	345	5.8
		Conc'n (ng/L)		**Conc'n (ng/L)**		**Conc'n (ng/L)**	
RR-06		NDMA-*d6*		DENA-*N2*		DPro-*d14*	
		Average	RSD (%)	Average	RSD (%)	Average	RSD (%)
	NDMA	757	10.3	788	7.5	764	7.2

Note: For **Tables A.4–A.11,** all data presented were quantified by isotope dilution using DPNA-*d14*. ND = not detected and NA = not applicable.

Table A.4. *N*-Nitrosamine comparison for sample RR-01 (chloraminated potable water) between University of Alberta and Gold Standard (GS) laboratories.

Laboratory	*N*-Nitrosamine (ng/L)							
	NDMA	MENA	DENA	DPNA	NMor	NPyr	NPip	DBNA
U Alberta	**4.1**	**ND**	**ND**	**ND**	**0.7**	**ND**	**ND**	**ND**
GS1	4.5	<2	<2	<2	<2	<2	<2	<2
GS2	4.2	<1	<1	<1	<1	<1	<1	<3
GS Mean	4.4	---	---	---	---	---	---	---
	% Difference							
UA / Mean (GS)	-5.2	NA	NA	NA	NA	NA	NA	NA

Table A.5. *N*-Nitrosamine comparison for sample RR-02 (RR-01 spiked at 12.7 ng/L) between University of Alberta and Gold Standard (GS) laboratories.

Laboratory	*N*-Nitrosamine (ng/L)							
	NDMA	MENA	DENA	DPNA	NMor	NPyr	NPip	DBNA
U Alberta	**16.9**	**13.4**	**13.0**	**11.9**	**16.7**	**12.6**	**11.2**	**12.6**
GS1	16.3	10.3	11.5	13.5	18.8	11.8	10.1	12.0
GS2	17.4	10.9	11.8	11.4	16.1	12.0	11.4	11.6
GS Mean	16.8	10.6	11.7	12.4	17.4	11.9	10.8	11.8
	% Difference							
UA / Mean (GS)	0.3	26.6	11.2	-4.4	-4.4	5.9	4.1	7.3

Table A.6. *N*-Nitrosamine comparison for sample RR-03 (RO effluent) between University of Alberta and Gold Standard (GS) laboratories.

Laboratory	*N*-Nitrosamine (ng/L)							
	NDMA	MENA	DENA	DPNA	NMor	NPyr	NPip	DBNA
U Alberta	**14.1**	**ND**	**ND**	**ND**	**3.0**	**ND**	**ND**	**ND**
GS1	14.3	<2	<2	<2	<2	<2	<2	<2
GS2	13.8	<1	<1	<1	2.3	<1	<1	<3
GS Mean	14.0	---	---	---	2.3	---	---	---
	% Difference							
UA / Mean (GS)	0.6	NA	NA	NA	29.1	NA	NA	NA

Table A.7. *N*-Nitrosamine comparison for sample RR-04 (chlorinated 2° wastewater effluent, reclamation plant) between University of Alberta and Gold Standard (GS) laboratories.

Laboratory	*N*-Nitrosamine (ng/L)							
	NDMA	MENA	DENA	DPNA	NMor	NPyr	NPip	DBNA
U Alberta	**163**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**
GS1	173	<2	<2	<2	3.6	<2	<2	<2
GS2	165	<1	<1	<1	4.0	<1	<1	<3
GS Mean	169	---	---	---	3.8	---	---	---
	% Difference							
UA / Mean (GS)	-3.7	NA	NA	NA	NA	NA	NA	NA

Table A.8. *N*-Nitrosamine comparison for sample RR-05 (RR-04 spiked at 376 ng/L) between University of Alberta and Gold Standard (GS) laboratories.

Laboratory	*N*-Nitrosamine (ng/L)							
	NDMA	MENA	DENA	DPNA	NMor	NPyr	NPip	DBNA
U Alberta	**537**	**378**	**362**	**337**	**353**	**369**	**342**	**345**
GS1	601	444	306	383	399	421	404	312
GS2	621	348	331	371	422	402	369	347
GS Mean	611	396	318	377	411	411	386	329
	% Difference							
UA / Mean (GS)	-12.0	-4.5	13.6	-10.6	-14.0	-10.3	-11.6	4.7

Table A.9. *N*-Nitrosamine comparison for sample RR-06 (chlorinated 3° wastewater effluent) University of Alberta and Gold Standard (GS) laboratories.

Laboratory	*N*-Nitrosamine (ng/L)							
	NDMA	MENA	DENA	DPNA	NMor	NPyr	NPip	DBNA
U Alberta	**764**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**
GS1	862	<2	<2	<2	8.2	7.6	<2	<2
GS2	842	<1	<1	<1	5.8	6.0	<1	<3
GS Mean	852	---	---	---	7.0	6.8	---	---
	% Difference							
UA / Mean (GS)	-10.3	NA	NA	NA	NA	NA	NA	NA

Table A.10. Method accuracy comparisons, low-level *N*-nitrosamine spike [Δ RR-02 minus RR-01 = 12.7 ng/L].

Laboratory	*N*-Nitrosamine (ng/L)							
	NDMA	MENA	DENA	DPNA	NMor	NPyr	NPip	DBNA
U Alberta	**12.7**	**13.4**	**13.0**	**11.9**	**15.9**	**12.6**	**11.2**	**12.6**
GS1	11.8	10.3	11.5	13.5	18.8	11.8	10.1	12.0
GS2	13.2	10.9	11.8	11.4	16.1	12.0	11.4	11.6
	% Accuracy							
U Alberta	100.4	105.7	102.0	93.6	125.2	99.0	88.2	99.5
GS1	92.5	81.1	90.6	105.9	147.6	92.5	79.5	94.1
GS2	103.9	85.8	92.9	89.8	126.8	94.5	89.8	91.3

Table A.11. Method accuracy comparisons, high-level *N*-nitrosamine spike [Δ RR-05 minus RR-04 = 376 ng/L].

Laboratory	*N*-Nitrosamine (ng/L)							
	NDMA	MENA	DENA	DPNA	NMor	NPyr	NPip	DBNA
U Alberta	**374**	**378**	**362**	**337**	**353**	**369**	**342**	**345**
GS1	427.5	444	306	383	395	421	404	312
GS2	456.0	348	331	371	418	402	369	347
	% Accuracy							
U Alberta	99.6	100.6	96.1	89.6	93.9	98.1	90.8	91.7
GS1	113.7	118.0	81.4	101.9	105.2	111.8	107.3	82.8
GS2	121.3	92.6	87.9	98.7	111.2	106.9	98.1	92.3

A.4 Conclusions

Participation in the WRF's analytical round-robin offered many opportunities to evaluate and to compare the *N*-nitrosamine method developed as part of this thesis project against the performances of several commercial, utility, and regulatory sector laboratories. As part of the round-robin testing, I was able to validate our high throughput, dual phase SPE extraction method combined with GC/MS PCI ammonia quantification by comparing my results with data generated by two gold standard laboratories. Additionally, I had the chance to interact and learn from many of the chemists, engineers, and administrators who were also involved in the project, enhancing my overall thesis project experience.

The high throughput SPE extraction and analytical quantification method developed as part of this thesis performed extremely well in the blinded analysis of *N*-

nitrosamines, at high and low concentrations in water and wastewater samples. Overall, University of Alberta data were highly comparable to the results from both Gold Standard laboratories. Percent differences between University of Alberta results and mean Gold Standard data were within ± 15% for all *N*-nitrosamines in all samples, with two exceptions. In one instance a University of Alberta result was more accurate for MENA in a low-level spiked sample compared to the Gold Standard laboratories, resulting in a % percent difference greater than 15%. The second exception was a low level result for NMor in RO effluent, where the University of Alberta measured 3 ng/L, GS2 measured 2.3 ng/L and GS1 was < 2 ng/L. University of Alberta average percent accuracies for the low- and high-level *N*-nitrosamine spikes were 102% and 95% respectively, comparable to the performances of GS1 and GS2.

The method employed throughout this thesis utilized bench-top analytical instrumentation, which is more commonly available and far less expensive to maintain and operate compared to the gold standard of high-resolution mass spectrometry, which is traditionally used for low level (ng/L) *N*-nitrosamine analysis in water. The highly comparable round-robin results between our method and the Gold Standard laboratory results confirms successful development of a selective, sensitive and affordable bench-top analytical method for *N*-nitrosamines, in drinking water as well as in more complex matrices such as wastewater effluents.

A.5 References

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Appendix B

N-Nitrosamine Raw Data

Note: All *N*-nitrosamine data in **Appendix B** are presented only as raw values and are not reported with an appropriate number of significant figures. Based on the precision and accuracy of the *N*-nitrosamine analytical method used throughout this thesis, no more than two significant figures are warranted when reporting *N*-nitrosamine values for use, as done throughout Chapters 2–6.

B.1 Chapter 2 Data

Table B.1. Performance of internal standard (IS) quantification method for a range of *N*-nitrosamine concentrations.

N-Nitrosamine	Exact Concentration (ng/L)						
	9.44	9.44	9.44	18.87	39.72	79.45	182.2
	Measured Concentration (ng/L)						
	1	2	3				
NDMA	9.5	9.2	9.6	19.3	34.4	78.8	182.5
MENA	8.8	8.9	9.1	18.7	31.9	77.3	176.1
DENA	9.3	9.7	9.6	18.6	30.0	69.2	168.1
DPNA	8.2	8.6	8.9	16.8	26.3	69.1	152.2
NMor	8.5	9.0	9.2	17.0	27.5	73.1	161.5
NPyr	7.7	8.1	8.5	16.6	26.7	71.9	158.0
NPip	7.3	8.2	7.8	15.5	26.1	65.0	143.1
DBNA	7.8	8.6	8.5	17.3	31.2	83.0	171.8

Table B.2. Performance of isotope dilution / surrogate standard (ID/SS) quantification method for a range of *N*-nitrosamine concentrations.

N-Nitrosamine	Exact Concentration (ng/L)						
	9.44	9.44	9.44	18.87	39.72	79.45	182.19
	Measured Concentration (ng/L)						
	1	2	3				
NDMA	9.4	8.6	8.4	18.1	44.1	79.7	182.8
MENA	8.6	8.2	7.8	17.7	41.8	80.4	181.7
DENA	9.2	9.1	8.4	18.3	41.1	75.5	182.6
DPNA	9.6	9.6	9.2	18.8	40.3	83.4	181.9
NMor	8.8	8.8	8.4	17.4	38.9	82.3	180.8
NPyr	7.8	7.8	7.6	16.9	38.3	82.2	179.9
NPip	9.4	10.0	8.9	18.8	42.7	83.7	182.3
DBNA	8.4	8.8	8.1	17.4	42.1	88.3	180.6

Table B.3. Absolute recovery determination using internal standard (IS) quantification method, for a range of spiked *N*-nitrosamine concentrations.

N-Nitrosamine	9.44	9.44	9.44	18.87	39.72	79.45	182.2	Mean	Rel Std	Std Dev	Min	Max
	Recovery (%)							(%)	(%)	(%)	(%)	(%)
NDMA	101	97	101	102	87	99	100	98.2	**5**	5.4	86.7	102.2
NDMA d6	117	123	132	120	87	109	110	113.9	**13**	14.4	86.5	132.1
MENA	94	94	96	99	80	97	97	93.9	**7**	6.3	80.3	98.9
DENA	99	103	102	99	76	87	92	93.9	**10**	9.8	75.5	102.8
DPNA	87	91	94	89	66	87	84	85.4	**11**	9.1	66.3	94.0
NMor	90	95	97	90	69	92	89	89.1	**10**	9.3	69.3	97.5
NPyr	81	86	90	88	67	91	87	84.2	**10**	8.0	67.3	90.5
NPip	78	87	82	82	66	82	79	79.2	**8**	6.7	65.6	86.8
DBNA	83	92	90	92	78	105	94	90.6	**9**	8.3	78.5	104.5

Table B.4. Percent accuracy for isotope dilution / surrogate standard (ID/SS) quantification method, for a range of spiked *N*-nitrosamine concentrations.

N-Nitrosamine	9.44	9.44	9.44	18.87	39.72	79.45	182.2	Mean	Rel Std	Std Dev	Min	Max
	Accuracy (%)							(%)	(%)	(%)	(%)	(%)
NDMA	99	91	89	96	111	100	100	98.1	**7**	7.3	88.7	110.9
NDMA d6	NA	NA	NA	NA	NA	NA	NA	NA	**NA**	NA	NA	NA
MENA	91	87	83	94	105	101	100	94.4	**8**	8.0	83.0	105.1
DENA	98	97	89	97	104	95	100	97.1	**5**	4.4	89.4	103.5
DPNA	102	101	98	100	101	105	100	101.0	**2**	2.3	97.7	105.0
NMor	93	94	89	92	98	104	99	95.6	**5**	4.9	89.1	103.6
NPyr	82	83	80	90	96	103	99	90.5	**10**	9.2	80.3	103.5
NPip	100	106	94	99	107	105	100	101.7	**5**	4.6	94.2	107.4
DBNA	89	93	86	92	106	111	99	96.6	**10**	9.2	85.9	111.1

Note: NA = not applicable, NDMA-*d6* was used as the surrogate standard for ID/SS.

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Table B.5. Quantification of *N*-nitrosamines detected in select Alberta drinking-water distribution system samples (July – September 2004).

Location	*N*-Nitrosamine	Concentration (ng/L)			Mean (ng/L)	Rel Std (%)	Std Dev (ng/L)
		1	2	3			
A1	NDMA	90.7	108.3	104.9	**101.3**	9	9.3
	NMor	0.7	0.8	---	**0.8**	9	0.1
	NPyr	3.8	4.8	3.0	**3.9**	23	0.9
A2	NDMA	63.4	80.6	66.7	**70.2**	13	9.1
	NMor	1.8	1.7	1.5	**1.7**	10	0.2
	NPyr	3.4	2.8	2.0	**2.8**	25	0.7
A3	NDMA	58.5	75.1	65.1	**66.2**	13	8.3
	NPyr	---	---	2.2	**2.2**	---	---
B	NDMA	7.9	---	8.1	**8.0**	1	0.1
C	NDMA	6.0	5.6	---	**5.8**	5	0.3
D	NDMA	4.2	5.1	5.5	**5.0**	14	0.7
E	NDMA	4.5	4.2	4.2	**4.3**	4	0.2
F	NDMA	3.7	3.2	4.5	**3.8**	17	0.7
G	NDMA	5.9	2.4	2.3	**3.5**	59	2.1
H1	NDMA	3.7	3.0	3.1	**3.3**	12	0.4
H2	NDMA	1.9	2.4	2.2	**2.2**	10	0.2
I	NDMA	5.1	4.6	4.9	**4.9**	6	0.3
	NMor	2.9	1.9	2.9	**2.6**	22	0.6
J	NDMA	4.8	4.7	4.9	**4.8**	3	0.1
K	NDMA	4.0	3.3	5.8	**4.3**	30	1.3
L	NDMA	3.3	3.5	3.6	**3.5**	4	0.1
M	NDMA	11.9	12.2	13.0	**12.3**	4	0.5
N	NDMA	3.5	4.5	3.6	**3.9**	14	0.5
O	NDMA	1.6	1.3	1.3	**1.4**	9	0.1
P	NDMA	2.9	1.2	1.6	**1.9**	47	0.9
Q	NDMA	NQ	NQ	NQ	---	---	---
R	NDMA	1.8	1.6	2.0	**1.8**	10	0.2
S	NDMA	1.8	1.6	1.6	**1.7**	7	0.1
T	NDMA	2.3	2.1	2.5	**2.3**	9	0.2

Table B.6. Surrogate standard (NDMA-*d6*) recoveries during *N*-nitrosamines quantification in select Alberta drinking-water distribution system samples (July – September 2004).

Location	*N*-Nitrosamine	Recovery (%)			Mean	Rel Std	Std Dev
		1	2	3	(%)	(%)	(%)
A1	NDMA-*d6*	64	78	78	**73.2**	11	7.9
A2	NDMA-*d6*	58	77	63	**66.1**	15	10.2
A3	NDMA-*d6*	66	87	75	**75.9**	14	10.6
B	NDMA-*d6*	88	---	89	**88.3**	1	0.6
C	NDMA-*d6*	87	80	---	**83.4**	6	4.9
D	NDMA-*d6*	82	76	88	**82.2**	7	6.0
E	NDMA-*d6*	91	81	85	**85.7**	6	4.9
F	NDMA-*d6*	84	82	82	**82.7**	1	1.2
G	NDMA-*d6*	76	78	63	**72.3**	12	8.4
H1	NDMA-*d6*	84	81	77	**80.7**	4	3.3
H2	NDMA-*d6*	70	77	88	**78.5**	11	8.6
I	NDMA-*d6*	84	78	79	**80.3**	4	3.1
J	NDMA-*d6*	85	91	90	**88.5**	4	3.4
K	NDMA-*d6*	91	86	91	**89.1**	3	3.1
L	NDMA-*d6*	99	98	103	**100.0**	2	2.3
M	NDMA-*d6*	94	87	94	**91.9**	4	4.0
N	NDMA-*d6*	89	97	86	**90.6**	7	6.1
O	NDMA-*d6*	80	79	87	**82.1**	5	4.5
P	NDMA-*d6*	72	62	69	**67.9**	8	5.2
Q	NDMA-*d6*	72	---	81	**76.3**	8	6.4
R	NDMA-*d6*	80	83	75	**79.1**	5	4.0
S	NDMA-*d6*	90	86	64	**79.6**	18	14.0
T	NDMA-*d6*	88	88	73	**83.1**	10	8.7

Table B.7. Performance of internal standard (IS) quantification method, determination of precision for *N*-nitrosamines spiked at 5.15 ng/L (n=7).

N-Nitrosamine	Concentration (ng/L)							Mean	Rel Std	Std Dev
	1	2	3	4	5	6	7	(ng/L)	(%)	(%)
NDMA	6.0	6.2	5.5	5.7	3.9	6.5	6.5	**5.7**	**16**	0.9
MENA	3.3	3.3	3.3	3.6	3.1	3.7	2.9	**3.3**	**8**	0.3
DENA	3.5	3.6	3.2	3.9	3.3	3.8	3.2	**3.5**	**7**	0.3
DPNA	4.3	4.8	4.2	5.6	4.2	4.8	3.8	**4.5**	**13**	0.6
NMor	4.0	4.2	3.4	4.5	3.9	4.5	3.4	**4.0**	**12**	0.5
NPyr	5.1	4.7	4.3	4.4	3.7	5.2	4.0	**4.5**	**12**	0.6
NPip	4.0	4.1	3.6	4.3	3.7	4.2	3.2	**3.9**	**10**	0.4
DBNA	4.8	4.7	4.1	5.0	4.8	4.9	3.5	**4.5**	**12**	0.5

Table B.8. Surrogate standard (NDMA-*d6*) recoveries during *N*-nitrosamine precision determination.

N-Nitrosamine	Recovery (%)							Mean	Rel Std	Std Dev
	1	2	3	4	5	6	7	(%)	(%)	(%)
NDMA	116	120	106	110	76	127	126	**111.5**	**16**	17.5
NDMA d6	63	67	60	69	62	75	68	**66.3**	**7**	4.8
MENA	64	64	63	70	60	71	57	**64.2**	**8**	5.1
DENA	67	70	63	75	65	74	63	**68.2**	**7**	5.1
DPNA	84	94	81	109	81	94	74	**88.2**	**13**	11.6
NMor	78	81	67	88	76	88	65	**77.6**	**12**	9.1
NPyr	100	91	83	85	73	101	77	**87.1**	**12**	10.7
NPip	77	80	70	83	72	81	63	**75.3**	**10**	7.4
DBNA	92	90	79	97	94	94	68	**87.9**	**12**	10.5

Table B.9. Performance of internal standard (IS) quantification method, absolute recoveries of laboratory fortified blanks at 10.29 ng/L, 19.72 ng/L, 39.43 ng/L and 102.64 ng/L.

N-Nitrosamine	LFB Concentration (ng/L)					
	10	20	40-A	40-B	100-A	100-B
NDMA	9.4	17.4	33.4	27.2	77.4	52.4
MENA	7.0	15.0	30.6	25.6	84.0	56.3
DENA	6.7	15.4	31.4	26.3	87.7	57.4
DPNA	8.0	18.7	36.8	31.1	104.7	69.8
NMor	8.1	18.4	33.8	29.5	99.8	66.3
NPyr	8.9	22.8	36.1	35.0	117.9	71.1
NPip	7.7	18.1	34.6	29.9	104.8	65.8
DBNA	8.6	20.5	38.9	33.7	115.4	75.1

Table B.10. Surrogate standard (NDMA-*d6*) recoveries during *N*-nitrosamine absolute recovery determination.

N-Nitrosamine	LFB and Surrogate Standard Recovery (%)					
	10	20	40-A	40-B	100-A	100-B
NDMA	91	88	85	69	75	51
NDMA d6	62	74	75	62	67	47
MENA	68	76	78	65	82	55
DENA	65	78	80	67	85	56
DPNA	77	95	93	79	102	68
NMor	79	93	86	75	97	65
NPyr	86	115	91	89	115	69
NPip	75	92	88	76	102	64
DBNA	84	104	99	85	112	73

Table B.11. Field and method blanks (July – September 2004).

N-Nitrosamine	Concentration (ng/L)									Mean	Rel Std	Std Dev
	1	2	3	4	5	6	7	8	9	(ng/L)	(%)	(%)
NDMA	2.4	2.3	1.4	1.3	3.7	2.9	2.6	0.9	3.2	**2.3**	47	1.1
	Recovery (%)									(%)	(%)	(%)
NDMA d6	56	73	62	60	76	72	77	59	61	**66.7**	12	8.1

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Table B.12. Detection of *N*-nitrosodimethylamine (NDMA) in chloraminated City A raw water.

Treatment	Concentration (ng/L)			Mean	Rel Std	Std Dev
	1	2	3	(ng/L)	(%)	(ng/L)
Raw Blank	1.6	1.2	1.0	**1.3**	**26**	0.3
1 mg/L	1.0	1.0	0.9	**1.0**	**6**	0.1
3 mg/L	2.2	1.5	1.2	**1.6**	**33**	0.5
4 mg/L	3.0	1.7	1.7	**2.1**	**35**	0.8
7 mg/L	9.0	8.4	6.4	**7.9**	**18**	1.4
12 mg/L	1.0	0.8	1.0	**0.9**	**16**	0.1
18 mg/L	0.6	0.7	0.9	**0.7**	**23**	0.2

Table B.13. Surrogate standard (NDMA-*d6*) recoveries in chloraminated City A raw water.

Treatment	Recovery (%)			Mean	Rel Std	Std Dev
	1	2	3	(ng/L)	(%)	(ng/L)
Raw Blank	81	57	52	**63.2**	**25**	15.6
1 mg/L	42	57	43	**47.3**	**18**	8.4
3 mg/L	50	57	44	**50.2**	**13**	6.5
4 mg/L	56	52	46	**51.2**	**10**	5.2
7 mg/L	56	58	41	**51.8**	**18**	9.2
12 mg/L	51	46	38	**44.9**	**14**	6.4
18 mg/L	45	47	40	**44.0**	**8**	3.7

Table B.14. Detection of *N*-nitrosodimethylamine (NDMA) in chloraminated City A partially-treated water.

Treatment	Concentration (ng/L) 1	2	3	Mean (ng/L)	Rel Std (%)	Std Dev (ng/L)
PT Blank	4.7	3.0	2.6	**3.4**	**32**	1.1
1 mg/L	8.4	6.9	11.5	**8.9**	**26**	2.3
3 mg/L	32.0	30.8	42.2	**35.0**	**18**	6.3
4 mg/L	39.9	33.7	46.7	**40.1**	**16**	6.5
7 mg/L	5.1	4.0	3.8	**4.3**	**17**	0.7
12 mg/L	4.2	2.6	2.3	**3.1**	**34**	1.0
18 mg/L	6.1	2.1	2.1	**3.4**	**68**	2.3

Table B.15. Surrogate standard (NDMA-*d6*) recoveries in chloraminated City A partially-treated water.

Treatment	Recovery (%) 1	2	3	Mean (ng/L)	Rel Std (%)	Std Dev (ng/L)
PT Blank	66	74	83	**74.2**	11	8.3
1 mg/L	62	74	86	**74.1**	17	12.2
3 mg/L	82	90	86	**86.0**	5	4.0
4 mg/L	85	74	77	**78.6**	7	5.7
7 mg/L	78	74	74	**75.5**	3	2.2
12 mg/L	67	72	66	**68.5**	5	3.5
18 mg/L	70	66	63	**66.3**	5	3.4

Table B.16. Detection of *N*-nitrosodimethylamine (NDMA) in chloraminated City B raw water.

Treatment	Concentration (ng/L)			Mean	Rel Std	Std Dev
	1	2	3	(ng/L)	(%)	(ng/L)
Raw Blank	0.8	---	0.7	**0.7**	---	---
1 mg/L	2.4	1.5	0.8	**1.6**	52	0.8
3 mg/L	1.1	0.8	1.1	**1.0**	13	0.1
4 mg/L	2.2	1.1	1.3	**1.5**	40	0.6
7 mg/L	1.8	1.4	1.4	**1.5**	15	0.2
12 mg/L	0.5	---	0.7	**0.6**	---	---
18 mg/L	0.6	---	0.8	**0.7**	---	---

Table B.17. Surrogate standard (NDMA-*d6*) recoveries in chloraminated City B raw water.

Treatment	Recovery (%)			Mean	Rel Std	Std Dev
	1	2	3	(ng/L)	(%)	(ng/L)
Raw Blank	57	---	53	**55**	---	---
1 mg/L	77	102	62	**80**	25	20.4
3mg/L	60	42	64	**55**	21	11.9
4 mg/L	61	49	55	**55**	11	6.3
7 mg/L	53	55	49	**52**	5	2.7
12 mg/L	38	---	55	**46**	---	---
18 mg/L	47	---	46	**47**	---	---

Table B.18. Detection of *N*-nitrosodimethylamine (NDMA) in chloraminated City B partially-treated water.

Treatment	Concentration (ng/L)			Mean	Rel Std	Std Dev
	1	2	3	(ng/L)	(%)	(ng/L)
PT Blank	2.3	1.0	1.2	**1.5**	49	0.7
1 mg/L	2.8	1.1	1.7	**1.9**	48	0.9
3 mg/L	2.0	2.0	1.8	**1.9**	5	0.1
4 mg/L	3.6	2.0	2.7	**2.8**	27	0.8
7 mg/L	2.5	1.7	2.4	**2.2**	19	0.4
12 mg/L	1.6	1.0	1.3	**1.3**	21	0.3
18 mg/L	2.1	1.0	---	**1.6**	---	---

Table B.19. Surrogate standard (NDMA-*d6*) recoveries in chloraminated City B partially-treated water.

Treatment	Recovery (%)			Mean	Rel Std	Std Dev
	1	2	3	(ng/L)	(%)	(ng/L)
PT Blank	59	65	60	**61.3**	5	3.3
1 mg/L	65	54	72	**63.7**	14	9.1
3mg/L	53	66	52	**56.8**	15	8.3
4 mg/L	64	64	72	**66.6**	7	4.8
7 mg/L	49	56	61	**55.4**	11	6.2
12 mg/L	50	58	62	**56.9**	11	6.1
18 mg/L	55	50	---	**52.2**	---	---

Table B.20. Absolute recovery and surrogate standard (NDMA-*d6*) recovery determination for spiked *N*-nitrosamines (9.42 ng/L) (March 3–5, 2005).

Sample	*N*-Nitrosamine	Concentration (ng/L)			Mean	Rel Std	Std Dev
		1	2	3	(ng/L)	(%)	(ng/L)
LFB Mar 3-5	NDMA	8.7	9.2	7.6	**8.5**	10	0.8
		Recovery (%)			(%)	(%)	(%)
	NDMA	92	98	81	**90.4**	10	8.7
	NDMA d6	88	98	84	**90.1**	8	7.2

Table B.21a. Absolute recovery and surrogate standard (NDMA-*d6*) recovery determination for spiked *N*-nitrosamines (9.42 ng/L) (March 9–11, 2005).

Sample	*N*-Nitrosamine	Concentration (ng/L)			Mean	Rel Std	Std Dev
		1	2	3	(ng/L)	(%)	(ng/L)
LFB Mar 9-11	NDMA	6.0	6.3	5.0	**5.8**	12	0.7
		Recovery (%)			(%)	(%)	(%)
	NDMA	64	67	53	**61.4**	12	7.2
	NDMA d6	64	73	53	**63.3**	16	10.1

Table B.21b. Reanalysis of absolute recovery and surrogate standard (NDMA-*d6*) recovery determinations for spiked *N*-nitrosamines (9.42 ng/L). Extracted March 9–11, 2005 and reanalyzed in May 2005.

Sample	*N*-Nitrosamine	Concentration (ng/L)			Mean	Rel Std	Std Dev
		1	2	3	(ng/L)	(%)	(ng/L)
LFB Mar 9-11	NDMA	7.7	8.0	6.6	**7.4**	10	0.8
Run in May		Recovery (%)			(%)	(%)	(%)
	NDMA	82	85	70	**78.8**	10	8.0
	NDMA d6	78	88	66	**77.3**	14	10.9

Table B.22. Absolute recovery and surrogate standard (NDMA-*d6*) recovery determination for spiked *N*-nitrosamines (9.42 ng/L) (April–May, 2005).

N-Nitrosamine	Concentration (ng/L)						Mean	Rel Std	Std Dev
	1	2	3	4	5	6	(ng/L)	(%)	(%)
NDMA	7.0	6.8	7.5	6.4	5.7	7.0	**6.7**	9	0.6
	Recovery (%)						(%)	(%)	(%)
NDMA	74	72	80	68	61	74	**71.7**	9	6.5
NDMA d6	63	66	79	73	62	73	**69.6**	10	6.7

Table B.23. Absolute recovery and surrogate standard (NDMA-*d6*) recovery determination for spiked *N*-nitrosamines (18.16 ng/L).

Sample	*N*-Nitrosamine	Concentration (ng/L)		Mean	Rel Std	Std Dev
		1	2	(ng/L)	(%)	(ng/L)
	NDMA	13.8	13.8	13.8	---	---
		Recovery (%)		(%)	(%)	(%)
	NDMA	76	76	76.2	---	---
	NDMA d6	78	76	76.9	---	---

Table B.24. Method blanks (March 3–5, 2004).

Sample	*N*-Nitrosamine	Concentration (ng/L)							Mean	Rel Std	Std Dev
		1	2	3	4	5	6	7	(ng/L)	(%)	(%)
OptBlk Mar 3-5	NDMA	0.7	0.7	0.6	---	---	---	---	0.7	4	0.0
		Recovery (%)							(%)	(%)	(%)
	NDMA d6	90	99	83	---	---	---	---	90.7	9	8.2

Table B.25. Method blanks (March 9–11, 2004).

Sample	*N*-Nitrosamine	Concentration (ng/L)							Mean	Rel Std	Std Dev
		1	2	3	4	5	6	7	(ng/L)	(%)	(%)
OptBlk Mar 9-11	NDMA	0.7	0.3	---	---	---	---	---	0.5	---	---
		Recovery (%)							(%)	(%)	(%)
	NDMA d6	70	67	---	---	---	---	---	68.5	---	---

Table B.26. Method blanks (April–May 2004).

Sample	*N*-Nitrosamine	Concentration (ng/L)							Mean	Rel Std	Std Dev
		1	2	3	4	5	6	7	(ng/L)	(%)	(%)
Apr-May Blks	NDMA	1.8	2.0	0.8	0.9	0.4	0.2	1.3	1.1	63	0.7
		Recovery (%)							(%)	(%)	(%)
	NDMA d6	85	93	79	92	62	61	82	79.0	17	13.0

B.4 *N*-Nitrosodimethylamine (NDMA) Data (July 2003–June 2005)

Table B.27. Detection of *N*-nitrosamines and surrogate standard (NDMA-*d6*) recoveries for samples collected July 4, 2003.

Location	*N*-Nitrosamine	Concentration (ng/L)		
		1	2	3
City A Finished	NDMA	6.2	5.5	—
		Recovery (%)		
	NDMA d6	85	83	—
City A Mid-Distribution	NDMA	52.5	42.3	—
		Recovery (%)		
	NDMA d6	83	79	—
City A End Distribution1	NDMA	62.2	55.1	—
		Recovery (%)		
	NDMA d6	81	81	—
City A End Distribution2	NDMA	89.0	67.0	—
		Recovery (%)		
	NDMA d6	98	72	—
City B Distribution	NDMA	ND	1.3	—
		Recovery (%)		
	NDMA d6	69	73	—

Table B.28. Detection of *N*-nitrosamines and surrogate standard (NDMA-*d6*) recoveries for samples collected September 15, 2003.

Location	*N*-Nitrosamine	Concentration (ng/L)		
		1	2	3
City A Finished	NDMA	13.74	---	---
	NMor	ND	---	---
	NPyr	0.22	---	---
		Recovery (%)		
	NDMA d6	67	---	---
City A Mid-Distribution	NDMA	57.12	---	---
	NMor	ND	---	---
	NPyr	1.1	---	---
		Recovery (%)		
	NDMA d6	109	---	---
City A End Distribution1	NDMA	62.08	---	---
	NMor	1.06	---	---
	NPyr	1.44	---	---
		Recovery (%)		
	NDMA d6	83	---	---
City A End Distribution2	NDMA	80.8	---	---
	NMor	0.706	---	---
	NPyr	2.1	---	---
		Recovery (%)		
	NDMA d6	83	---	---
City B Distribution	NDMA	0.96	---	---
	NMor	ND	---	---
	NPyr	ND	---	---
		Recovery (%)		
	NDMA d6	73	---	---

Table B.29. Detection of *N*-nitrosamines and surrogate standard (NDMA-*d6*) recoveries for samples collected April 29, 2004.

Location	*N*-Nitrosamine	Concentration (ng/L)		
		1	2	3
City A Finished	NDMA	6.2	5.5	---
		Recovery (%)		
	NDMA d6	85	83	---
City A Mid-Distribution	NDMA	52.5	42.3	---
		Recovery (%)		
	NDMA d6	83	79	---
City A End Distribution1	NDMA	62.2	55.1	---
		Recovery (%)		
	NDMA d6	81	81	---
City A End Distribution2	NDMA	89.0	67.0	---
		Recovery (%)		
	NDMA d6	98	72	---
City B Distribution	NDMA	ND	1.3	---
		Recovery (%)		
	NDMA d6	69	73	---

Table B.30. Detection of *N*-nitrosamines and surrogate standard (NDMA-*d6*) recoveries for samples collected July 19, 2004.

Location	*N*-Nitrosamine	Concentration (ng/L)		
		1	2	3
City A Mid-Distribution	NDMA	58.5	75.1	65.1
	NMor	ND	ND	ND
	NPyr	ND	ND	2.2
		Recovery (%)		
	NDMA d6	66	87	75
City A End Distribution1	NDMA	63.4	80.6	66.7
	NMor	1.8	1.7	1.5
	NPyr	3.4	2.8	2.0
		Recovery (%)		
	NDMA d6	58	77	63
City A End Distribution2	NDMA	90.7	108	105
	NMor	0.7	0.8	ND
	NPyr	3.8	4.8	3.0
		Recovery (%)		
	NDMA d6	64	78	78
City B Distribution	NDMA	4.2	5.1	5.5
	NMor	ND	ND	ND
	NPyr	ND	ND	ND
		Recovery (%)		
	NDMA d6	82	76	88

Table B.31. Detection of *N*-nitrosamines and surrogate standard (NDMA-*d6*) recoveries for samples collected December 20, 2004.

Location	*N*-Nitrosamine	Concentration (ng/L)		
		1	2	3
City A Finished	NDMA	11.4	---	---
		Recovery (%)		
	NDMA d6	68	---	---
City A Mid-Distribution	NDMA	82.9	---	---
		Recovery (%)		
	NDMA d6	67	---	---
City A End Distribution 1	NDMA	113	---	---
		Recovery (%)		
	NDMA d6	67	---	---
City A Prefilter Plant	NDMA	2.2	---	---
		Recovery (%)		
	NDMA d6	77	---	---
City A Pre-UV Plant	NDMA	4.1	---	---
		Recovery (%)		
	NDMA d6	63	---	---
City A Post-UV Plant	NDMA	6.2	---	---
		Recovery (%)		
	NDMA d6	76	---	---

Table B.32. Detection of *N*-nitrosamines and surrogate standard (NDMA-*d6*) recoveries for samples collected February 25, 2005.

Location	*N*-Nitrosamine	Concentration (ng/L)		
		1	2	3
City A Finished	NDMA	4.2	4.2	4.5
		Recovery (%)		
	NDMA d6	57	60	56